THE INFLUENCE OF THE USE OF BUTTER ON THE FREEZING PROPERTIES OF ICE CREAM MIX*

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In some sections of the United States milk fat for ice cream can be purchased more cheaply in the form of butter than in the form of sweet cream. As a result butter is quite commonly used for this purpose and the ice cream produced is said to compare favorably in quality with that containing sweet cream. Clutter (1) and Wright (2) studied some of the factors which influence the freezing properties of ice cream mixes and found among other things that mixes made with butter did not swell as much nor as rapidly as those made with cream. They also observed that butter mixes were more viscous than cream mixes. No adequate explanation seems to have been offered to account for these observations.

This paper records the results of experimental work designed to study the differences in the freezing properties of ice cream mixes made with cream and butter.

EXPERIMENTAL

A quantity of fresh 40 per cent cream was held for several hours at 45° to 50°F. One-half of the cream was then churned and worked into butter. This butter and the remainder of the cream were then used in the preparation of separate mixes, each of which contained 10 per cent fat, 10 per cent M.S.N.F., 14.5 per cent sugar and 0.5 per cent gelatin. Dry skimmilk was used as the source of the solids-not-fat required in addition to those furnished by the cream.

After pasteurization each mix was homogenized at 3000 pounds pressure, cooled and aged for twenty-four hours at 35°F. All mixes were then frozen in a forty-quart brine freezer. The

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swell and consistency of the ice cream in the freezer were determined at minute intervals. This procedure was repeated, using in all, four lots of cream each of which was from a different source.

A summary of the freezing properties of the mixes made with cream showed that a swell of 90 per cent was obtained 4.5 minutes after the brine was turned off, whereas the butter mixes averaged 8.2 minutes before this selected overrun was reached. An average maximum swell of 101 per cent was obtained with the mixes
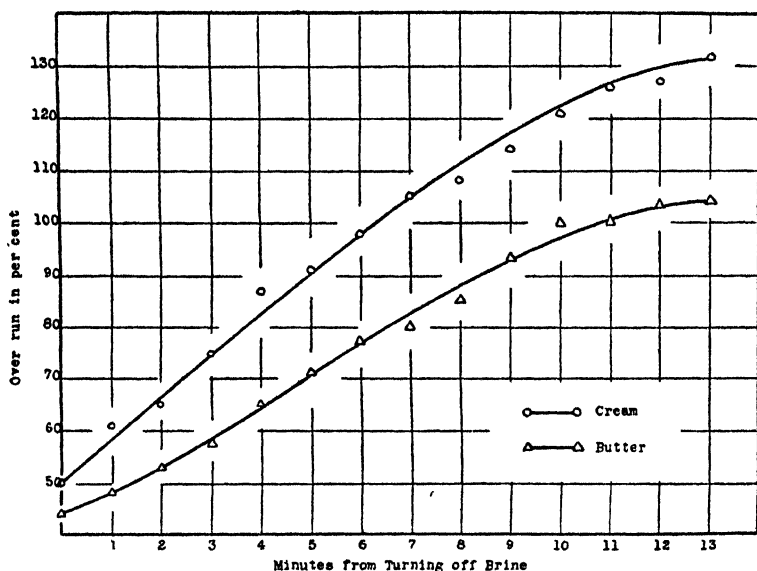

FIG. 1. SWELL DEVELOPMENT OF MIXES MADE WITH CREAM AND WITH BUTTER

containing cream, while the butter mixes had an average maximum swell of 76 per cent. The swell development of both the cream and butter mixes is shown graphically in figure 1. It is evident that the mixes made with butter did not whip as rapidly nor to as high a maximum as the mixes made with cream.

Cream may be considered to be a mixture of butter and buttermilk. In the butter mix the buttermilk portion of the original cream was not incorporated, since it was discarded during the manufacture of the butter. It was necessary then, to increase

slightly the amount of dry skimmilk in the butter mix to maintain the desired serum solid standard of 10 per cent. The cream and the butter mixes differed only in this substitution of dry skimmilk for the buttermilk solids of the cream lost in the churning process. It seems logical to assume therefore, that the unequal freezing properties of these mixes might be attributed either to the quantity of dry skimmilk used or to the amount of buttermilk solids present in each mix. The work of Lucas and Roberts (11) indicates that the amount of dry skimmilk used in a well
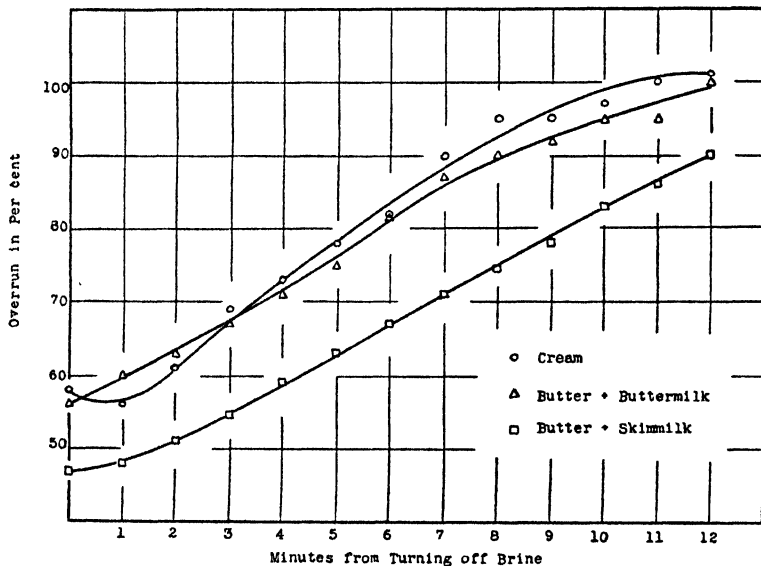

FIG. 2. SWELL DEVELOPMENT OF MIXES MADE WITH BUTTERFAT FROM VARIOUS SOURCES

proportioned mix containing 10 per cent fat has no significant influence on the whipping properties. It would seem, therefore, that the difference noted in the freezing properties was due to the amount of buttermilk solids, rather than due to the quantity of dry skimmilk.

If the more desirable freezing properties of the cream mix could be attributed to the presence of the buttermilk, it would follow that a butter mix would exhibit about the same freezing properties

as a cream mix if the buttermilk produced during churning were incorporated in the mix with the butter. Such a batch was made and compared with one mix containing cream and a second mix made with butter in the manner already described. The milkfat was, of course, obtained from the same source of milk. This procedure was repeated, using in all, three separate lots of cream. The mixes in this experiment contained: fat 12 per cent, M.S.N.F. 11 per cent, sugar 14.5 per cent and gelatin 0.5 per cent. Data concerning the behavior of these three mixes in the freezer are shown in table 1 and in figure 2.

TABLE 1

Freezing properties and basic viscosity of mixes made with and without buttermilk solids

| | FREEZING PROPERTIES | | |
DESCRIPTION OF MIX	Time to reach 90 per cent swell*	Maximum swell obtainable with desired consistency	BASIC VISCOSITY OF AGED MIX
	minutes	*per cent*	*cp.*
Cream..............................	7	92	89
Butter and buttermilk..................	8	90	83
Butter.............	12	75	121

* Time from moment of turning off brine.

The data of table 1 indicate that butter mixes had a higher basic viscosity than the cream mixes which contained more buttermilk solids. This is in accordance with the statements of Clutter (1) and Wright (2). It is also apparent that the freezing properties of the mix in which the buttermilk solids were absent were inferior to those of the mixes containing these solids. It would seem from these results that the coalescing of the fat globules during the churning process and the subsequent reëmulsification of the fat in making the ice cream had but little effect on the swell development. Apparently buttermilk and cream contain some factor which is wholly or partially lacking in butter which is able to affect to a marked extent the viscosity and the freezing properties of the mixes.

DISCUSSION

Clutter (1) and others observed that high basic viscosity is often associated with low overrun and that an important factor in determining basic viscosity is the extent of the aggregation of the fat globules. These results have been confirmed by the experiments reported here and by work as yet unpublished by the author. Examination of the mixes made in the course of these experiments indicated a marked difference in the clumping of the fat globules. The fat in those mixes containing more of the buttermilk portion of the cream did not gather in large aggregations during the homogenizing process, but this clumping did occur in the other mixes. This suggests the presence of a

TABLE 2

Lecithin in milk products

MATERIAL	AVERAGE PERCENTAGE OF LECITHIN
Milk..	0.0447
Cream..	0.1981
Skimmilk...	0.0165
Buttermilk	0.1302
Butter (raw sweet cream).................................	0 0723
Butter (pasteurized ripened)	0.0433

material capable of stabilizing the emulsion of the fat in those mixes containing the buttermilk portion of the cream.

Examination of the available information brings out the fact that this material might be lecithin. Table 2 from the work of Chapman (3) and Supplee (10) shows that buttermilk and cream contain 13 to 20 times as much lecithin as skimmilk. Palmer and Samuelson (4) found that the butter milk from washed cream contained a mixture of phosphatides. Thurston and Petersen (5) present further evidence in support of this theory. They determined the lecithin content of buttermilk made by churning creams of various fat contents and showed that the richer the cream in fat the greater the quantity of lecithin found in the buttermilk. Dornic and Daire (6) suggest that lecithin is asso-

ciated with the butterfat, probably in the form of a film around the fat globules, and that the coalescing of the fat globules during the churning process brings about the mechanical separation of the lecithin from the fat, causing it to remain in the buttermilk.

The probable difference in the lecithin content of the mixes with and without the buttermilk portion of the cream suggests that lecithin may be the factor causing the marked difference in the freezing properties.

The theory that the presence of lecithin in ice cream mix improves the whipping quality, is further supported by the statements of Dahle (7), Turnbow and Raffetto (8), Wright (2) and others, that the addition of dry egg yolk to the mix has a beneficial effect on the swell. Dahle says that egg yolk "is a good emulsifying agent and a very small amount will stabilize a large amount of fat." According to Haas and Hill (9) egg yolk contains 9.4 per cent of lecithin.

SUMMARY

1. Experimental data is presented to show that the use of butter in place of cream as the source of fat in ice cream mixes produces a mix of inferior freezing qualities.

2. Butter mixes differ from cream mixes in that they do not contain the cream serum or "buttermilk solids" of the cream.

3. Data recorded by previous workers indicate that the lecithin content of cream and buttermilk is considerably higher than that of skimmilk and butter.

4. It is suggested that the lecithin incorporated in ice cream with cream, buttermilk or egg yolk may be responsible for the differences in the freezing properties observed when this material is partially lacking in the mix.

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THE PHYSIOLOGICAL EFFECT OF PITUITARY EXTRACT (POSTERIOR LOBE) ON THE LACTATING MAMMARY GLAND*

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Extracts of the pituitary gland (hypophysis cerebri) have long been known to have an effect on the blood pressure (Oliver and Schafer (19)) inducing a marked rise following injection. Subsequently there was observed contractile action on smooth muscle and a diuretic effect. Howell (13) first pointed out that the active principal was a constituent of the posterior lobe. Herring (12) however, produced evidence indicating that while the active principal is present in the posterior lobe, it is a product of the cells of the pars intermedia.

The profound effect on the lactating mammary gland of infundibulin (the active principle of the posterior part of the hypophysis) was first demonstrated by Ott and Scott (20) in 1910. Upon the injection of an extract into a lactating goat, the flow of milk started in about one minute, reached its maximum in four minutes, and then fell rapidly. These results were quickly confirmed with lactating cats and dogs by Schafer and Mackenzie (22), in dairy cattle by Gavin (7), and in man by Schafer (23).

In cattle Gavin (7) observed a rapid flow of milk to the lower parts of the udder, the teats usually being distended within ten to fifteen minutes of injection. No change was noted in the daily yield or composition of the milk.

Schafer and Mackenzie (22) noted that the effectiveness of repeated doses was greatly reduced or entirely absent. Hammond (10) verified this observation and tried without effect to overcome the supposed immunity by enlarged doses. Differing from Gavin's results, Hammond observed an increase in the percentage

of fat in the milk following the injection. Simpson and Hill (25) working independently, confirmed the observations of Hammond.

All investigators are agreed that the injection of extracts from the posterior lobe of the pituitary brings about a rapid flow of milk when the teat has been excised or makes immediately available a certain quantity of milk which could not ordinarily be removed by milking or nursing.

There is, however, a considerable difference of opinion in regard to the mode of action. It was thought by the early workers that pituitrin was a true galactogogue stimulating the synthesis of milk. Others claimed that the extract acted on the mammary gland in some manner resulting in the expulsion of the formed milk from the individual secreting cells or from the alveoli or both.

Experimental evidence in favor of the secretory theory of action was advanced by Simpson and Hill (25) who injected barium chloride, a strong smooth muscle stimulant, into a goat and found no increased milk flow. They conclude, therefore, that the action is on the secretory mechanism of the gland. Maxwell and Rothera (17) found that the injection of the extract into goats which had only recently been milked dry caused considerable additional milk to become available. They state, "It seemed to us unlikely that such a large increase could be due to simple pressure on the gland caused by contraction of its muscular element." Further, it was shown that following an injection there was an increased milk pressure lasting seventeen and one-half minutes in a goat and at least forty minutes in a cow. They conclude that the effect of pituitrin is the same as suckling causing a true secretion of milk. More recent work which will be described later indicates that little if any actual secretion of milk occurs as a result of suckling or milking.

Hammond (10) concluded as a result of his work that "an injection of pituitary extract is to cause the immediate secretion of the milk (stored in the gland) which would otherwise have been drawn off at the next milking." Schafer (23) gives the following observation of his patient "that though there was more milk than

usual for the baby for the meal following the injection, I had to wait a long time before I could feed him again."

The most pertinent objection to the secretory theory is advanced by Schafer (24). He pointed out that a second injection administered immediately after a gland has been completely emptied is without effect. It is then quite impossible to get a drop of milk from it, although if the action is on the secreting mechanism a free flow should be obtained. To produce the reaction one must either use a gland which is full or one not completely depleted; and if it has been depleted, one must wait until some secretion has accumulated within the alveoli.

MODE OF ACTION

Several theories have been advanced in explanation of the mode of action of pituitrin.

It has been suggested that the mode of action is through alterations produced on the circulation of blood. It was shown by Howell (13) that injections of the extract slowed the heart rate but increased the blood pressure. Both effects are less marked than in the case of epinephrin (extract of the adrenal medulla) but they persist for a longer time. Geiling (8) states further that the respiratory rate is quickened, but is interspersed with periods of cessation of breathing in unanesthetized dogs. Repeated injections have not only little or no blood pressure-raising action, but may produce a diametrically opposite effect—a depressor response.

A further action of the extract is its stimulating effect on most of the smooth muscles of the body. The intestine and bladder are made to contract. There is also considerable oxytocic activity through the stimulating effect upon the uterine musculature. The difference in the mode of action of pituitary extract and epinephrin is described by Howell (14). "While epinephrin acts mainly at least on plain muscle innervated by the sympathetic autonomics and gives contractions or relaxation according as the nerve-fibers are motor or inhibitory, the hormone from the posterior lobe of the pituitary gland appears to act directly on the muscle and to cause contraction or increased tone in all cases."

Because of the general similarity of some of the physiological reactions of pituitary extract and epinephrin, the comparative action on the lactating mammary gland is of extreme interest. Hammond and Hawk (11) found that following injections of adrenalin (epinephrin) in the goat there was no immediate action on milk secretion, whereas there was a secondary effect indicated by a decrease in the amount of milk produced for a period of a day following its injection.

Similarly Simpson and Hill (25) injected barium chloride, a strong muscle stimulant, into a goat and found no increased milk flow.

The absence of stimulation of milk flow with these extracts which are known to react upon smooth muscle immediately raises the question as to the presence of smooth muscle or related structures surrounding the alveoli or ducts. Unfortunately this problem is still in a contraversial state with little general agreement. Benda (1) found in the club shaped ends of the 'gland tubules between the low cuboidal epithelium and the membrana propria, cells which he considered identical to the muscle cells of the sweat glands. He was of the opinion that these structures possess contractility and play a part in the physiology of secretion. Zacher (30), Unger (29), Spampani (27), Lacroix (15), Christ (3), Lenfers (16), Bertkau (2) and others maintain that they have observed either smooth muscle cells, or basket cells (myoepithelium) which have contractile properties associated with the alveoli and ducts of the mammary gland.

On the other hand, Hammond (10) and more recently Dieckmann (4) advance the idea that the cells thought to be myoepithelium are, instead, cells which surround the capillaries of non-epithelial origin.

Instead of a muscular action Hammond (10) believes that the pituitary extract acts directly upon the glandular epithelium causing the contraction of the cells and expulsion of the secretion. Sections of the gland following pituitary extract injection showed alveoli distended with milk and the epithelium cells flattened. The epithelial cells of the control were columnar and protruded into the lumens, which were small, and contained only limited

quantities of milk. In a few places, however, the alveoli were filled, and here the epithelial cells were flattened.

In spite of the work of Simpson and Hill (25) showing that barium chloride had no effect on the mammary gland Schafer (24) still maintains that the "complete emptying of a gland which obtains after an adequate injection can best be explained by supposing the existence of contractile tissue in the walls of the alveoli."

In support of this theory Schafer presents a microphotograph showing the alveoli of two adjacent mammary glands of a lactating cat. In one of these the nipple had been excised so that the milk could flow away freely, in the other gland the nipple was left intact, the resistance furnished by its tissues being always sufficient to prevent an outflow of secretion under these circumstances. After injection, the alveoli in the gland with the excised teat showed small irregular lumens, whereas the others were expanded and full of milk. He concludes, "It seems to me impossible to suppose that the vis a tergo produced by the action of the secreting cells in forwarding the fluid into the alveoli could produce such a picture as that of the emptied gland here exhibited."

A further hypothesis as to the mode of action of pituitrin is one suggested to Schafer (24) by Professor T. H. Milroy. It is postulated that the alveoli are highly extensible and elastic; that the secretion accumulates within them owing to a tonic contraction of plain muscle in the ducts, preventing its exit; and that pituitrin produces inhibition of this contraction so that elastic reaction of the alveoli can produce the outflow which is then obtained. The result of the experiment with barium which Simpson and Hill describe would fit in with this inhibitory hypothesis, while it appears to contraindicate the contraction hypothesis.

Schafer maintains that the emptying of the alveoli due to retraction of stretched elastic fibres in their walls, consequent on inhibition of plain muscle in the ducts is ingenious, but receives no support from analogy; nor has it been shown that the mammary gland is better provided with elastic fibres than other secreting glands. In fact, in the cat and rat gland there are

practically no elastic fibres in the walls of the alveoli nor between the alveoli, and these fibres are not especially numerous in the interstitial tissue of the gland.

Evidence from another source in favor of the above hypothesis is presented by Emmerson (5) who injected a 2 per cent solution of apothesin (a local anasthetic) around the inguinal nerves of a lactating dairy cow. In one case it was noted that although the cow was milked but a few hours previously, milk began to drop from the ends of the teats for a period of 45 minutes. In a second case, a cow producing 21.5 pounds of milk daily was remilked twenty minutes after an injection and produced 3.7 pounds of milk.

OBJECT OF THE INVESTIGATION

The review of experimental work indicates that pituitrin is one of the few animal extracts which has a definite physiological effect on the mammary gland. It, therefore, becomes of great importance in connection with studies on milk secretion and the physiological activity of the mammary gland during the milking process.

The discharge of milk at the time of milking noted by Nüesch (18) Maxwell and Rothera (17), and Tgetgel (28), as indicated by an increase in pressure within the cistern system, appears to be the result of a nervous reflex. The stimulus of milking usually sets in motion the phenomena called "letting down" of the milk extensively studied by Tgetgel (28). The nervous stimulus may be brought to the gland through the activity of motor nerves acting on the smooth muscles. It is possible, however, that one of the normal functions of the pituitary gland, which is so closely connected with the nervous system, is to regulate the discharging phase of milk secretion. If this were the case, the nerve paths would lead to the pituitary gland causing the discharge of the hormone which would in turn bring about the well known change in the gland.

PRESENTATION OF EXPERIMENTAL DATA

Our first objective in beginning experimental work with pituitrin[1] was to standardize the extract in terms of its physio-

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[1] Lilly's Pituitary Extract, Surgical, Double U. S. P. strength was used.

logical activity in the lactating dairy cow. The statement of Hammond (10) that the extract causes the immediate secretion of the milk stored in the gland was thought to offer such a standard. The plan adopted was to milk the cow regularly at 5 a.m. and 5 p.m. each day. On Monday and Wednesday of each week the cow was remilked at 5:30 p.m. On Friday immediately after the regular milking 2 cc. of the extract were injected subcutaneously. The cow was then remilked at 5:30 p.m. Each week the volume of extract was increased by 2 cc.

TABLE 1

The effect of varying amounts of pituitrin

PITUITRIN	30 MINUTES REMILK (2 DAY AVERAGE)		30 MINUTES REMILK AFTER INJECTION		12 HOURS MILKING AFTER INJECTION		24 HOURS MILKING AFTER INJECTION	
	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat
cc.	cc.	per cent	cc.	per cent	pounds	per cent	pounds	per cent
2	101.0	5 0	233.0	5.45	12 0	3.0	13.4	3.1
4	93 5	4.7	504 0	5.90	10.0	2 4	14.2	3.1
6	85.0	5.4	870 0	6.10	9.5	1 6	16 0	3.6
8	123.3	6.0	812.0	7.10	7.5	1.9	15.7	3.4
10		7.2	803.0	7.10	5.2	1.4	15.3	2.8
12		6 0	916.0*	7.90	4 7†	6 0	15.6	3.1

* Milked at ten-minute intervals for thirty minutes.
† Milked hourly for six hours following injection.
440 cc. of milk is equal to 1 pound.

The effect of increasing amounts of pituitrin on the amount of milk which could be obtained thirty minutes after the previous milking is shown in table 1 and figure 1. It will be seen that the yield of milk increases until 6 cc. were injected. Slightly more milk was obtained with 12 cc., but in this case the cow was milked at ten-minute intervals for thirty minutes. The experimental animal weighed about 1200 pounds. From these observations it is concluded that approximately 0.5 cc. of pituitrin per 100 pounds live weight produces the maximum physiological effect so far as the expulsion of milk is concerned.

The percentage of fat shows a gradual rise as the amount of extract increases.

TEMPORARY INHIBITION OF MILK SECRETION

Not only does pituitrin exert an immediate influence on the gland following an injection, but there appears to be a secondary effect on the subsequent secretion of milk much greater than that which can be accounted for by the milk immediately removed. It will be noted from figure 2 that the amount of milk and the
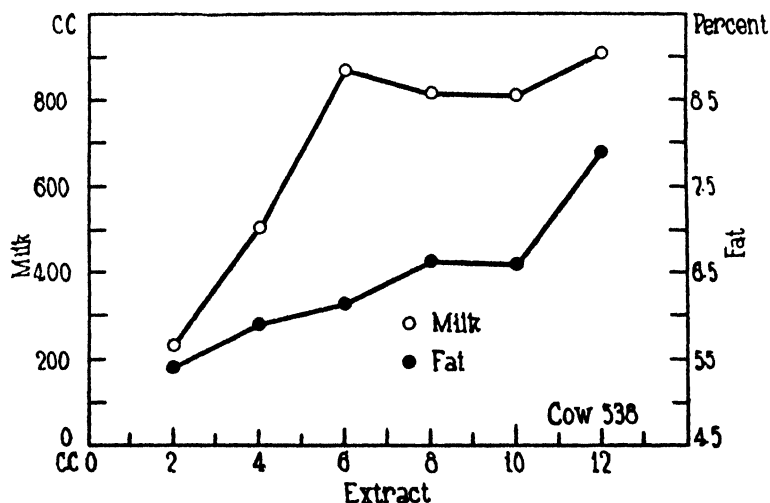

FIG. 1. THE EFFECT OF INCREASING AMOUNTS OF PITUITRIN ON THE AMOUNT OF MILK WHICH COULD BE OBTAINED THIRTY MINUTES AFTER THE PREVIOUS MILKING

The maximum yield of milk was obtained with 6 cc. The percentage of fat continued to increase with the larger injections. The animal weighed about 1200 pounds and was producing about 30 pounds of milk per day. From these observations, it is concluded that 0.5 cc. of pituitrin per 100 pounds live weight produces the maximum physiological effect in forcing out the residual milk from the udder.

percentage of fat obtained twelve hours after the injection decreased as the volume of pituitrin was increased. This phenomena can be explained on the basis that the extract inhibits the normal secretion of milk for a time following its injection, and that the duration of the period is in proportion to the size of the dose. At the rate of decline observed, it would appear that about 18 cc. of extract should entirely inhibit milk secretion for twelve hours.

That the inhibition is temporary, however, is indicated by the yield and composition of the milk obtained twenty-four hours after the injection. At that time the normal yield was obtained in every case. In this connection it may be of some interest to note that the monthly rate of milk secretion was distinctly on the decline as the animal was in the eighth month of lactation when the injections were begun. The monthly milk yield (thirty-one-

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It will be noted that both the yield of milk and the percentage of fat decline, as the dose increases. These results may be explained by assuming that the extract inhibits the normal secretion of milk for a time following its injection.

day month) beginning in May, 1927, were as follows: May, 1244 pounds; June, 1360 pounds; July, 1408 pounds; August, 1318 pounds; September, 1111 pounds; October, 932 pounds; November, 835 pounds. The first injection was made on December 23. The total production for December was 833 pounds, for January 881 pounds (the maximum injections were made at this time) and for fifteen days in February at the rate of 862 pounds of milk.

During the entire period of injection covering approximately two months, the monthly production was maintained approximately at the rate of secretion at the time the experiment was started.

To further study the inhibitory effect of pituitrin, the cow was injected with 12 cc. of pituitrin and milked at hourly intervals for a period of 6 hours. The results are shown in table 2 and figure 3. For comparison, the normal hourly secretion of milk during the two preceding check periods are included.

TABLE 2

The effect of pituitrin injections on subsequent hourly milkings

TIME	NORMAL HOURLY SECRE-TION—CHECK I		NORMAL HOURLY SECRE-TION —CHECK II		12 CC. PITUITRIN AFTER 5.30 MILKING	
	Milk	Fat	Milk	Fat	Milk	Fat
p.m.	cc.	per cent	cc.	per cent	cc.	per cent
5:30	6,600	3 50	6,644	3 50	6,292	2.90
6:00	113	5 90	96	6.10	916	7.80
7:00	55	3 00	56	3.70	218	3.40
8:00	575	2 80	398	2.85	225	3 10
9:00	250	2.70	937	3.20	210	2.90
10:00	1,200	2.95	348	3.05	193	2.80
11:00	62	2 65	342	2.75	146	2.25
12:00	850	2.25	743	2 55	151	1.85
Total (excluding first re-milk period.	2,992		2,824		1,143	
Grand total .	3,105		2,920		2,059	

Under normal conditions the fluctuation in the yield of milk obtained at hourly intervals is rather large due, it is believed, to the fact that after short intervals the stimulus of milking does not produce the usual pressure changes in the udder which permits the removal of the milk present.

It will be noted that after pituitrin is injected, the normal mechanism regulating the discharge of milk is no longer effective and the milk appears to become available to the milker as it is secreted. From these data it appears that the effect of pituitrin on the mammary gland is to greatly inhibit the rate of secretion.

The fact that the inhibition is rather uniform throughout the entire period is contrary to our expectation. It was believed that little or no milk would be secreted for the first several hours
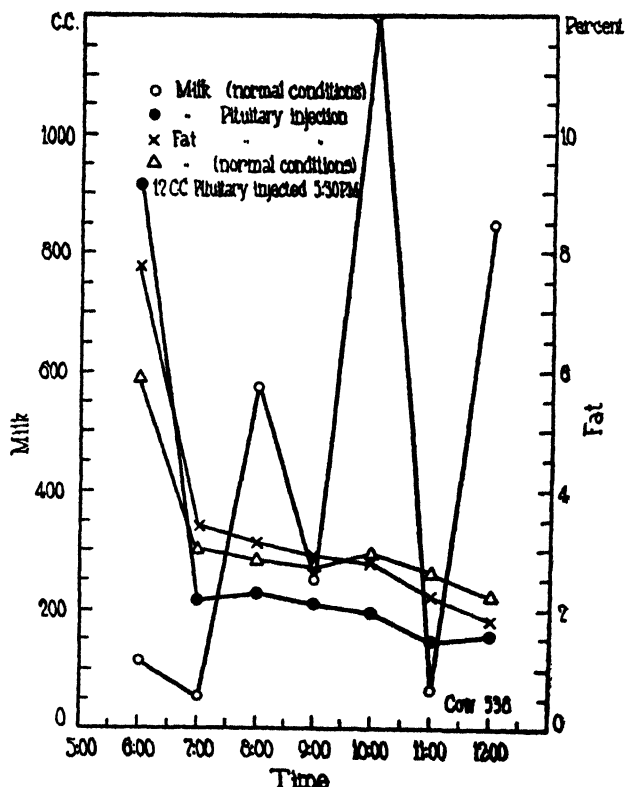
FIG. 3. THE EFFECT OF 12 CC. OF PITUITRIN ON THE YIELD AND COMPOSITION OF MILK DURING THE FOLLOWING 6 HOURS IS SHOWN IN COMPARISON WITH THE NORMAL PRODUCTION

It will be noted that under normal conditions, there are large fluctuations in the hourly production of milk whereas following the injection of the extract the rate of secretion is greatly inhibited but the violent fluctuations are absent.

with a gradual increase as the effect of the extract wore off. On the contrary, there was a gradual decline in the milk yield from first to last. It is possible that the removal of milk may give a false picture of the nature of the inhibition.

The percentage of fat declined rather uniformly in both the normal and experimental periods, but was the more marked after the injection.

In a further effort to determine the length of time the extract inhibits milk secretion, the cow was milked for eighteen hours following an injection. The effect of an injection of 10 cc. of pituitrin on the hourly milk production is presented in

TABLE 3

The length of time the udder was affected by pituitrin

TIME	MILK	FAT	SPECIFIC GRAVITY
a.m.	*cc.*	*per cent*	
5:30	6,820 0	4.05	1.0320
6:30	530.0	6.25	1.0250
7:30	313.0	3.15	1.0282
8:30	238 0	2 05	1.0315
9:30	130.0	2.15	1.0319
10:30	88.0	1.80	1.0341
11:30	54.0	2.25	1.0346
p.m.			
1:30	105.0	1.50	1.0370
2:30	252.0	1.75	1 0374
3:30	495.0	1.15	1.0384
4:30	462.0	0 95	1 0385
5:30	1,880.0	2 55	1.0366
6:30	70.0	2.05	1.0345
7:30	134.0	2.25	1.0339
8:30	94.0	2 05	1.0349
9:30	1,040.0	1 95	1.0376
10:30	176.0	1 85	1 0352
11:30	230.0	2.25	1 0358

table 3. The effect was apparently lost in about nine hours, as the variability in the hourly yields of milk noted in previous control milkings was again noticeable at this time. It will be noted that at 5:30 a.m. at the usual milking time, a large yield was obtained.

REACTION TIME

In order to ascertain the time required for the subcutaneous injection of pituitrin to become effective, the cow was remilked at

10 minute intervals for thirty minutes following injections of 6 and 12 cc. of the pituitrin.   The results are shown in table 4.

When 6 cc. of pituitrin were injected, the largest amount of milk was available at the end of twenty minutes.   With the injection of 12 cc., the largest amount was available at the end of ten minutes.   The highest percentages of fat were obtained in the first and second intervals.

TABLE 4

Time required for pituitrin to take effect

AMOUNT OF PITUITRIN	5.30 TO 5.40		5:40 TO 5:50		5:50 TO 6:00		TOTAL
	Milk	Fat	Milk	Fat	Milk	Fat	
cc.	cc.	per cent	cc.	per cent	cc.	per cent	cc.
6	133	5.7	375	5.5	150	4 9	658
12	660	8 1	155	8.2	101	6.4	916

COMPOSITION OF MILK

The change in the percentage of fat in the milk following pituitrin injection has already been presented.   Complete analysis of a number of samples of milk have also been made. The results are shown in table 5.   The "normal milk" refers to the milk obtained at the usual milking period, the milk at "remilking" is that obtained thirty minutes later as a normal check. The influence of pituitrin on the composition will be noted in the comparison with the "remilk" samples.

The results obtained confirm the observations of Hammond (10), and Simpson and Hill (25), namely, that pituitrin affects chiefly the fat content of the milk.

DISCUSSION OF RESULTS

These experiments confirm the work of previous investigators in that pituitrin has been clearly demonstrated to have important physiological properties in connection with the discharge of milk from the mammary gland.   Whether the posterior lobe of the pituitary gland plays a part in the normal discharge of milk at milking time is still an open question.   Our work was concerned with the effect of increasing amounts of pituitrin causing a

discharge of the "residual" milk in the udder after the normal milking period.   That pituitrin does not cause a secretion of milk but rather a discharge of milk already formed in the udder, is indicated by the work of Gaines and Sanmann (6) and Gowen and Tobey (9) who found more lactose in the udder of the cow at milking time than was obtained at a given milking.   The average excess of lactose found by Gowen and Toben represented an

TABLE 5

Composition of milk as affected by pituitary extract (cow 538)

DESCRIPTION OF SAMPLE	TOTAL SOLIDS	ASH	LAC-TOSE	TOTAL PRO-TEIN	CASEIN	FAT
	per cent	per cent	per cent	per cent	per cent	per cen
Normal milk January 4, 5:30 p.m.....	11 37	0 40	3.71	3.41	2 46	2 7
Milk at remilking 6:00 p.m.	12 09	0 35	3 35	3.13	2 23	4 9
Normal milk, January 6, 5:30 p.m.. .	11 72	0 50	4.18	3 22	2.62	2 8
Milk, 6 cc. pituitrin 6:00 p.m.. ...	13.65	0 48	4 12	2 87	2.04	6.1
Normal milk, January 9, 11, and 13, p.m.	13 05	0.69	4 71	3.32	2.52	3 3
Milk at remilking, January 9 and 11	15 16	0 67	4 09	3 32	2 39	6.3
Milk, 8 cc. pituitrin, January 13..	15 41	0 67	4 07	2 55	1 79	7.1
Normal milk, January 14	11 40	0.72	4.07	3.67	2 84	1.9
Normal milk, January 16 and 18 .	12 36	0.73		3.28	1.53	3.5
Milk at remilking, January 16 ..	15 40	0.61	4 33	3 00	2 36	7 6
Milk at remilking, January 18	14 55	0 62	3.21	3.06	1 40	6 8
Normal milk, January 20	14 55	0 62	3 21	3.06	1 40	6.8
Milk at remilking, 10 cc. pituitrin January 20.	15 69	0.67	4 12	3 03	1 85	7.6
Milk, January 21, a.m.	10 61	0 67	4 23	3 57	2 86	1 4

The chemical analyses were made by the Department of Agricultural Chemistry.

average equivalent of 2.1 pounds of milk remaining in the udder after the cow's udder was believed to be milked dry.

The 4 trials using from 6 to 12 cc. showed that an average of 1.93 pounds of milk were obtained as a result of pituitrin injection.   These facts are believed to offer further evidence that pituitrin is not a true galactogogue stimulating the synthesis of milk but rather acts upon the discharging mechanism making it possible to obtain the "residual" milk in the cow's udder.

If pituitrin acted only to cause a more complete discharge of

milk, it would be expected that at the subsequent milking, the yield of milk would be reduced an equivalent amount. These data show, however, that as the amount of extract increased there was a greater and greater reduction in the yield of milk twelve hours later, far exceeding the amount obtained at the remilking periods.

These data indicate a second important physiological property of pituitrin never before demonstrated, namely a temporary inhibition of milk secretion varying in intensity with the amount of extract injected. Subsequent hourly milking indicated that the inhibition of milk secretion is not complete during the first few hours even when sufficient pituitrin is injected to reduce the total twelve-hour milk yield to one-third of that of the normal production. The temporary decrease in milk secretion may be due to a reduction in the flow of blood through the udder due to a contraction of the vascular system. Another possibility is that the secretion of milk may be inhibited by the pressure in the udder due to the contraction of the smooth muscle elements. If the later explanation were correct, the withdrawal of milk at hourly intervals would not give a true picture of the inhibition. A better measure of the effect would be the measurement of the pressure at hourly intervals without removal of any milk. Further observations are required to settle this point.

Reviewing all the evidence, the writers are inclined to the theory that pituitrin is not a galactoguge but rather acts on the mechanism normally effective during the milking process. The excessive amounts injected make possible the discharge of a certain additional amount of "residual" milk still present in the udder at the close of the normal milking act. While the work presented has not contributed to the question of the mode of action of pituitrin or of the normal discharge of milk, we are inclined to believe that contractile elements in the walls of the alveoli and ducts furnish the vis a tergo observed.

SUMMARY

1. The maximum physiological effect indicated by a discharge of "residual" milk was obtained by approximately one-half cubic centimeter of pituitrin per 100 pounds live weight of the cow.

2. A temporary inhibition of milk secretion which varied in intensity with the amount of pituitrin injected was indicated by the yield of milk obtained at the next regular milking.

3. The inhibitory effect of 10 cc. of pituitrin lasted approximately ten hours.

4. The time required for the pituitrin to become effective varied with the size of the injection. The greatest yield of milk was obtained at the end of ten minutes when 12 cc. were injected whereas twenty minutes were required when only 6 cc. were used.

5. The data confirm the work of previous investigators in indicating that probably fat alone of the constituents of milk is influenced by this extract.

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COLOR OF EVAPORATED MILKS*

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The color of evaporated milk is of considerable commercial importance since it is one of the fundamental characteristics by which the consumer judges the product. Some of the various factors concerned in the production of color are recognized in a general qualitative way by the producer. No data, however, are available dealing with the factors concerned in the production of color of evaporated milks from a quantitative standpoint.

It has been the purpose throughout this work to study the effect of various steps in the manufacturing process upon the color of the resulting product, using therein for comparisons, standards of exact known color values.

THE MUNSELL COLOR SYSTEM

The Munsell (2) color system, based upon the psychological fact that color has three attributes; namely, hue, brilliance, and chroma, has been described by Cleland (1), and by Nickerson (3). Troland (4) defines these terms as follows: "Hue is that attribute of certain colors in respect of which they differ characteristically from a gray of the same brilliance and which permits them to be classed as reddish, yellowish, greenish or bluish." "Brilliance (or value) is that attribute of any color in respect of which it may be classed as equivalent to some member of a series of grays ranging between black and white." "Chroma (or saturation) is that attribute of all colors possessing a hue which determines their degree of difference from a gray of the same brilliance." Figure 1 illustrates the relationship of the three color attributes.

Hue. Hue is indicated by the horizontal radii of the color solid and is divided into five principal hues, red (R), green (G), yellow (Y), etc., and five intermediate hues, yellow-red (YR), green-

25

yellow (GY), etc. These ten are known as standard hues and each is further subdivided into ten hues.

Brilliance. The vertical axis of the color solid, as shown in figure 1, consists of gradations from white to black through a series of grays. For each position of the hue circle in relation
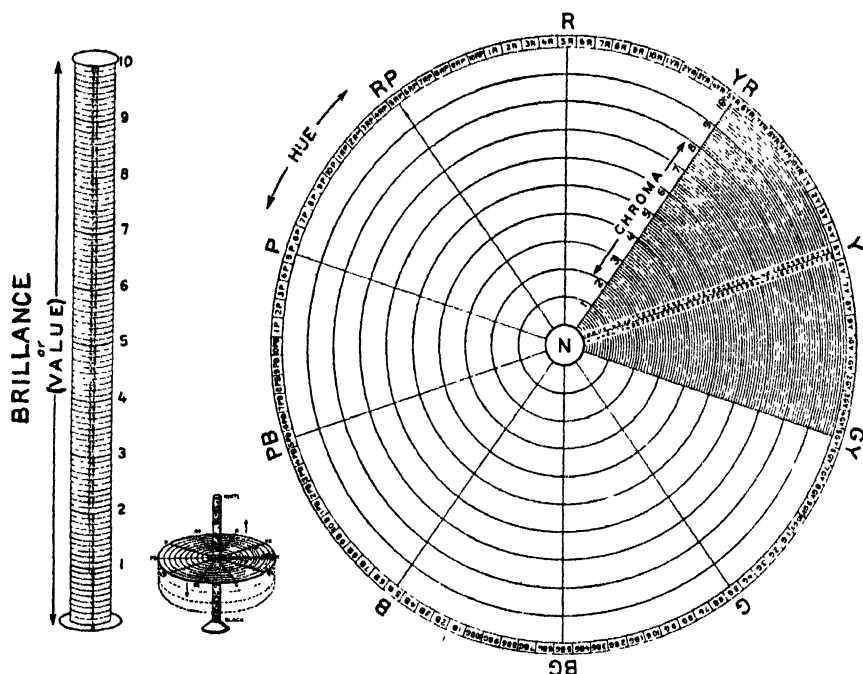
FIG. 1. RELATIONSHIP OF BRILLIANCE, CHROMA, AND HUE ACCORDING TO MUNSELL

to the brilliance scale (small diagram in figure 1) the brilliance of the colors varies.

Chroma. Chroma, or saturation, is indicated on the radii of the color solid, ranging from gray of a certain brilliance at the center to maximum color strength at the circumference. In recording data, brilliance and chroma are usually written as a fraction: Brilliance / Chroma, 4/5, 6/8, etc.

APPARATUS

The Munsell apparatus is provided with discs made to represent the principal and intermediate hues as yellow, yellow-red, etc., at each step in chroma and brilliance. Each disc is marked as to notation of color attributes. The index also provides discs ranging from black to white.

Two or more hue discs and neutral discs are spun on a motor shaft at a speed high enough to combine the colors into a single color. A surface, the color of which is to be measured, is placed beside the discs. A slit in each disc from circumference to center (Maxwell discs) allows for overlapping of the discs and the proportions of the visible sections can be adjusted until there is a perfect match between the object under test and the resolved color. Constant lighting conditions are essential for accurate work. If standard artificial daylight is not available, then North light is next to be preferred. The distance from the eye to the discs should be about 4 feet and the areas exposed to the eye should be equal.

DATA

After the colors are matched the composite disc is super-imposed on a disc with a perimeter graduated into 100 divisions and readings of the exposed areas of each disc used are taken. The sum of the hue disc and the neutral discs should equal 100.

The following formulae have been developed for general use in calculating hue, brilliance, and chroma (3):

$$\text{Hue: } z - \frac{\Sigma (A_z P_z)}{\Sigma (A_z P_z) + \Sigma (A_x P_x)} (z - x)$$

$$\text{Brilliance: } \sqrt{\frac{A_1 B_1^2 + A_2 B_2^2 + \ldots}{100}}$$

$$\text{Chroma: } \frac{A_1 C_1 + A_2 C_2 + \ldots}{100}$$

where A = area, H = hue, B = brilliance, C = chroma, x = 1st hue, clockwise, z = 2nd hue, clockwise, P = Power $(B \times C)$. Notation: $H^B/_C$.

EXPERIMENTAL

Color standards

A normal evaporated milk was prepared and samples sterilized at various temperatures and for various lengths of time until a series of ten samples were obtained ranging in color from that of non-sterilized evaporated milk to a deep brown.

Permanent standards were then prepared from $CaCO_3$ (precipitated chalk) suspensions to which were added varying amounts
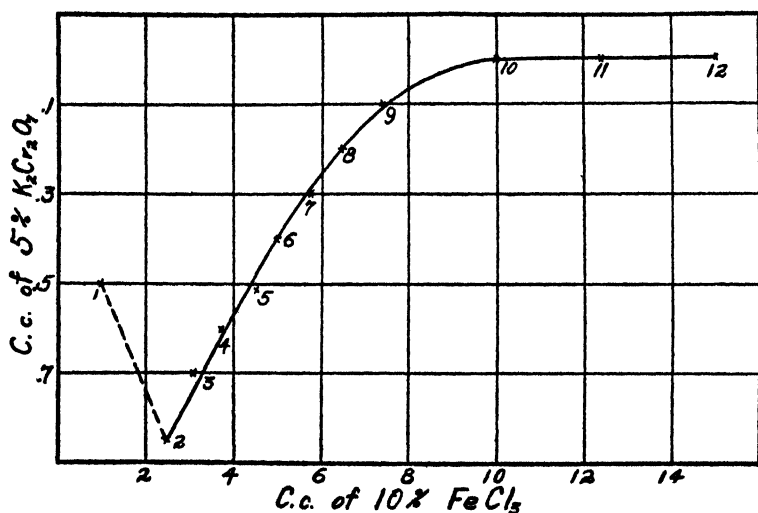
FIG. 2. AMOUNTS OF $K_2Cr_2O_7$ AND $FeCl_3$ SOLUTIONS ADDED TO 200 CC. OF A $CaCO_3$ SUSPENSION (1:5) TO PREPARE EACH COLOR STANDARD

Standard no. 1—to match unsterilized milks. Standards nos. 2 to 12—to match sterilized milks.

of solutions of 10 per cent $FeCl_3 \cdot 6H_2O$ and 5 per cent $K_2Cr_2O_7$ until these suspensions seemed to match in color the various milk samples previously prepared. These two sets of standards were then subjected to comparison by direct color measurements by the Munsell system. It was early noted that the artificial standards darkened slightly upon aging. Measurements from time to time indicated that equilibrium was reached at the end of approximately one month.

New standards of the CaCO$_3$ suspensions were prepared and adjusted in color with the FeCl$_3$ and K$_2$Cr$_2$O$_7$ solutions until their readings at the end of a month's storage checked approximately those of the evaporated milks. Each of the standards was placed in a 250-cc. flat-faced clear glass bottle and sealed to prevent loss of moisture. In making comparisons similar bottles were used
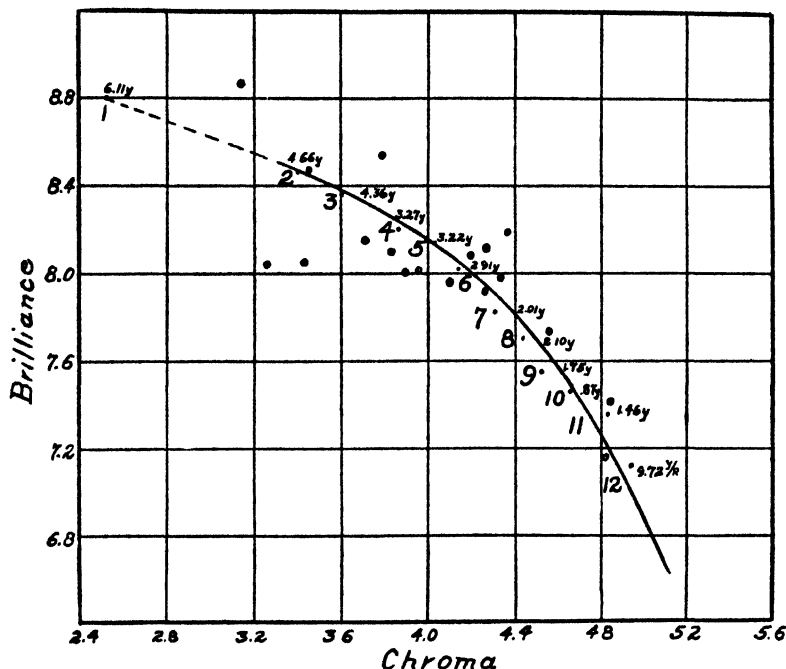
FIG. 3. MEASUREMENTS OF COLOR OF EVAPORATED MILKS AND ARTIFICIAL STANDARDS ACCORDING TO THE MUNSELL SYSTEM

● = commercial samples.   ⊙ = experimental samples   • = artificial standards. The hue is inserted for each standard.

for the milk samples. A rack to hold the bottles with a wide slit opening to expose a flat surface of uniform size of each sample greatly facilitated comparisons. Comparisons are best made in diffused light from a north window.

In figure 2 are shown the amounts of solutions of 5 per cent K$_2$Cr$_2$O$_7$ and 10 per cent FeCl$_3$·6H$_2$O which were added to each

200 cc. of a $1:5CaCO_3$ in water suspension to produce a standard. The addition of $FeCl_3$ solution imparts a brown coloration to the suspension while the addition of the $K_2Cr_2O_7$ imparts a yellow and slightly greenish hue.

TABLE 1

Effect of time and temperature of sterilization upon color and final pH of evaporated milk

Sterilization temperature 108°C.

					TIME			
	Check	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes	30 minutes	35 minutes
pH	6.36	6 32	6 29	6.28	6 25	6 22	6.20	6.17
Color	1−	1+	1++	2−	2	2+	3	3+

Sterilization temperature 112°C.

	Check	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes	30 minutes	35 minutes
pH	6.36	6.28	6.24	6.23	6.22	6.20	6.15	6.12
Color	1−	1+	2−	2	2+	3+	5−	6−

Sterilization temperature 116°C.

	Check	4 minutes	8 minutes	12 minutes	16 minutes	20 minutes	24 minutes	28 minutes	32 minutes
pH	6.36	6.28	6.22	6.19	6.14	6.10	6.07	6.03	6.02
Color	1−	1+	2−	2	3	4	5	6	7+

Sterilization temperature 120°C.

	Check	3 minutes	6 minutes	9 minutes	12 minutes	15 minutes	18 minutes	21 minutes	24 minutes	27 minutes	30 minutes
pH	6.36	6.23	6.20	6.18	6.15	6.12	6.06	6.02	6.00	5 95	5 90
Color	1−	1+	2−	3−	4−	5	6	7	8	9	10−

In figure 3 are given the readings upon the milks and the artificial standards prepared in terms of the Munsell notations. Brilliance is plotted against chroma, while the variations in hue for each standard are inserted in the figure.

For the particular milk samples first used the artificial standards prepared were a good imitation. Other milks prepared subsequently indicated considerable variation in readings in the region of lower chroma values, and it seems probable that standards Nos. 1, 2, and 3 (especially 1 and 2) should be of greater brilliancy to check the milk samples more perfectly. For practical purposes, however, these standards are seldom used since practically every evaporated milk sterilized sufficiently to keep well corre-
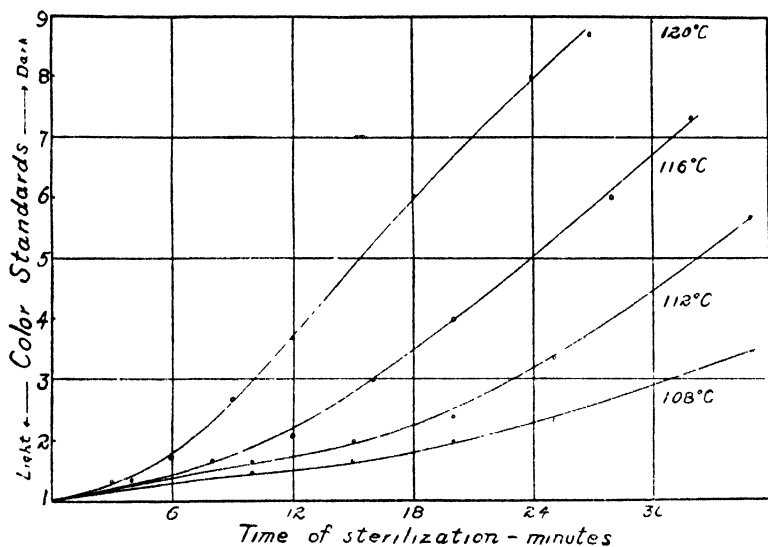
FIG. 4. EFFECT OF TIME AND TEMPERATURE OF STERILIZATION UPON THE COLOR OF EVAPORATED MILKS

sponds in color to some standard of greater color depth, an average commercial milk having a color matching standards No. 4 or 5.

The twelve standards selected for use in this work parallel the shades of evaporated milk from unsterilized (No. 1) to heavily sterilized samples (No. 12). In the data given the deviations from any standard are reported as + or − that number, the degree of color difference between each number in the following sequence being considered of equal value: 1, 1+, 2−, 2+, etc.

Factors influencing color variations of evaporated milks

Time and temperature of sterilization. The time and temperature of sterilization are the most important factors influencing the color of the evaporated product. An increase of exposure to heat is accompanied not only by an increase in color but also by a corresponding lowering in the final pH of the product. These
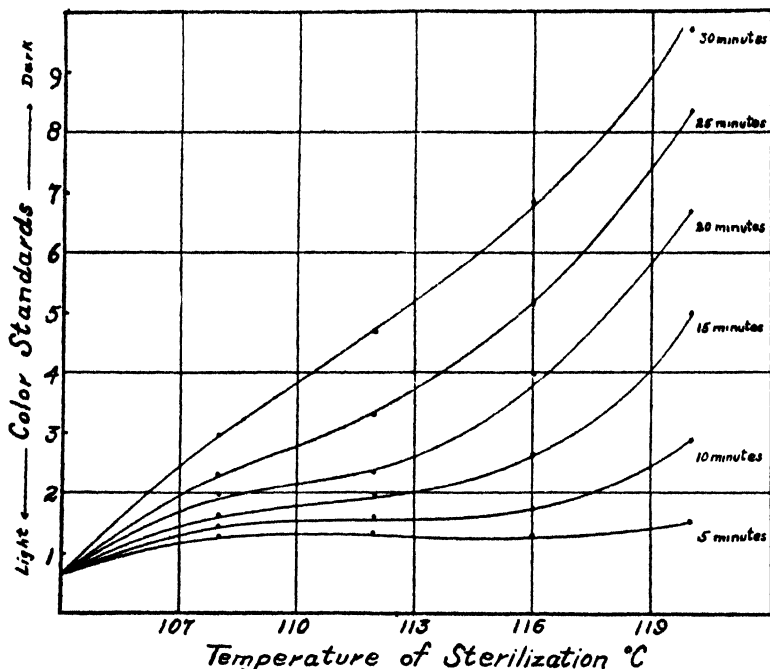
FIG. 5. EFFECT OF TIME AND TEMPERATURE OF STERILIZATION UPON THE COLOR
OF EVAPORATED MILKS

relationships are shown by representative data, upon the same milk, given in table 1.   Data from this table showing the effect of time and temperature of sterilization on color are plotted in figure 4.   This figure indicates that the production of color is not a direct function of time but that the reaction is of a catalytic nature.   With respect to temperature, for any chosen time of sterilization, the relationship is of the same nature, as shown in figure 5.

Although there is a continuous change in the H-ion concentration of the sterilized milks, as shown in table 1, their color is not a direct function of their pH. This is illustrated in figure 6.

Time of forewarming. The length of time of forewarming increases the exposure to heat and hence should cause the milk to reach the true logarithmic phase of color production more rapidly when it is sterilized. Table 2 indicates, however, that within the
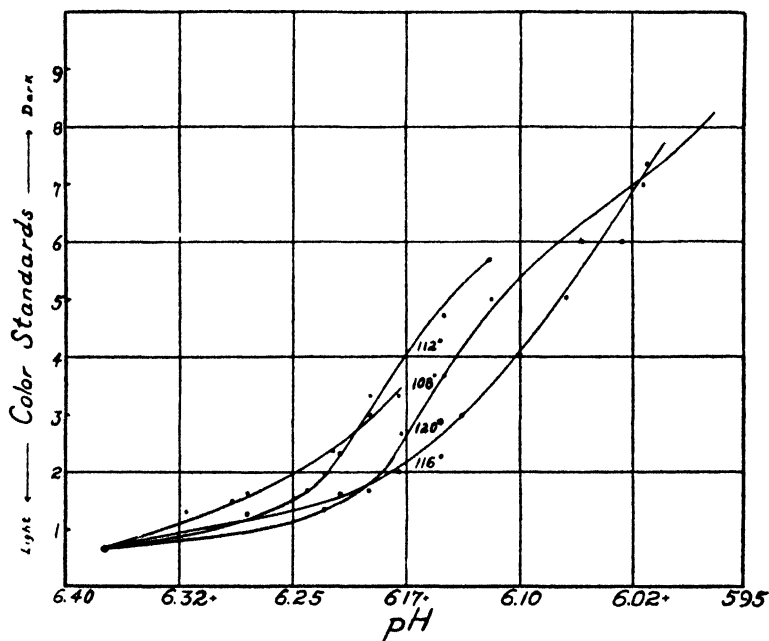
FIG. 6. RELATIONSHIP OF COLOR AND pH OF MILKS DURING STERILIZATION AT DIFFERENT TEMPERATURES

time of forewarming usually applied there is no great change in the color of the resulting product. For extremely long periods (thirty minutes) the color is greatly affected.

Homogenization. Homogenization breaks up the larger fat globules and hence diminishes the color of the product. Some of the results obtained are given in table 3.

Addition of neutralizers. At times the addition of neutralizers to evaporated milk influences the color of the product when ster-

ilized. Additions of small amounts of phosphates, citrates, and lime water failed to produce any change in color upon the milks used. However, a darkening of color was observed upon the addition just previous to sterilization of various amounts of a solution of 10 per cent sodium bicarbonate. A gradual deepening

TABLE 2

Effect of time of forewarming upon the color of the finished product

TIME OF STERILIZATION AT 118°C.	TIME OF FOREWARMING AT 95°C.	COLOR AFTER STERILIZATION
minutes	*minutes*	
Check—no sterilization	1	1+
	15	1+
	30	1++
12	1	5
	15	5+
	30	7-
18	1	8+
	15	9+
	30	11-

TABLE 3

Effect of homogenization upon the color of the finished product

TIME OF STERILIZATION AT 118°C.	COLOR AFTER STERILIZATION	
	Not homogenized	Homogenized at 3000 pounds
minutes		
Check—no sterilization	1	1-
5	2	2-
10	3+	3
15	6	5
20	8	6
25	10+	8

of color was observed in every case where this salt was used as a neutralizer. However, when the added $NaHCO_3$ was neutralized with lactic acid to the pH of the milk before sterilization, there was no measureable darkening in color of the final product as shown in figure 7. Data for a number of different milks to which $NaHCO_3$ was added are also plotted in figure 7.

Storage. Storage of sterilized evaporated milks results in an increase in their color. Samples of normal homogenized evaporated milks sterilized at 118° for 13 minutes were prepared and stored at the following temperatures: 5°, 10°, 20°, 30°, and 37°C. These samples were compared with the color standards at various intervals up to three months' time. No change in color
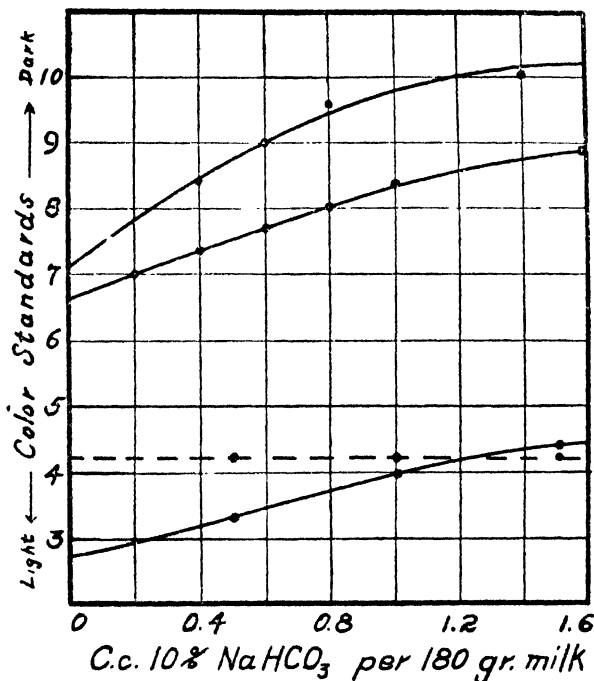
FIG. 7. EFFECT OF NaHCO₃ UPON COLOR OF EVAPORATED MILKS

——— NaHCO₃ added. ---------- Sodium lactate, at same pH as milk added. Constant time and temperature of sterilization for each series.

was noted in the milks stored at 5°C. Progressive darkening with increase in temperature and time of storage was noted in the other milks. After the sixth week of storage those milks held at 30° and 37°C. would no longer match in color any of the artificial standards. A color change of a different nature from that observed during sterilization was taking place.

Actual color measurements made with the Munsell equipment upon milks stored for three months at various temperatures showed this color change which occurred during storage to be one of chroma only, the brilliance and hue changing but slightly. This change in chroma resulted in a milk having a yellowish-green appearance. In figure 8 are plotted data representing the color change of a normal evaporated milk during sterilization at 118°C. and after storage for 3 months at 10°, 20°, 30°, and 37°C.
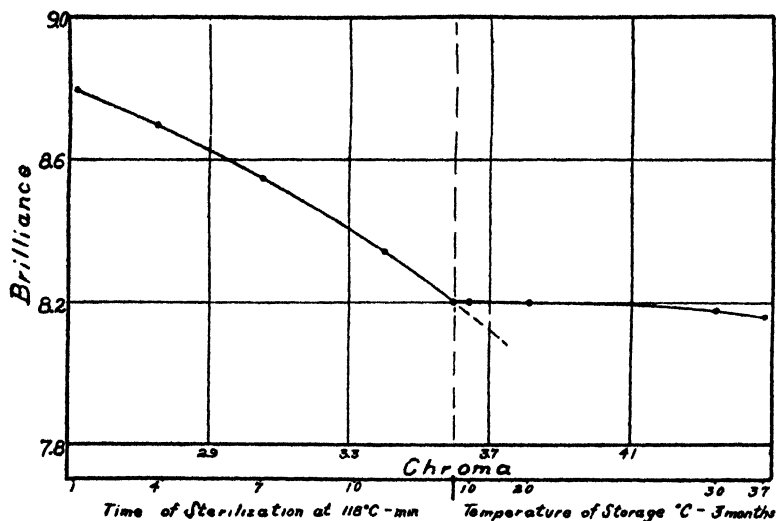
FIG. 8. CHANGES IN THE COLOR OF EVAPORATED MILK AS EFFECTED BY STERILIZA-
TION AND THREE MONTH'S STORAGE AT VARIOUS TEMPERATURES

DISCUSSION

The use of $CaCO_3$ as a base, with $FeCl_3$ and $K_2Cr_2O_7$ to impart color, makes a permanent and satisfactory material for evaporated milk color standards. To obtain standards of uniform differences and a numerical expression for each color, the standards were read and recorded according to the Munsell color system. These standards may be reproduced with a limit of error of one-half to one degree of difference, a degree here being considered the color difference between any two consecutive standards. Difficulty was encountered in obtaining standard

the color of which conformed exactly to the color shades of milks from various sources. With a constant milk supply, the pigments will not vary enough to cause difficulty when the exact shade has been obtained. The $FeCl_3$ increases the red-brown color while the $K_2Cr_2O_7$ imparts a green-yellow color. By proper blending of these two chemicals in the white base any desired evaporated milk color may be duplicated. Three to four degrees, however, should be allowed for darkening during the first month before equilibrium is reached.

The time-temperature relationship during sterilization is the most important factor affecting the color of evaporated milk. Since this relationship closely approximates a constant in industrial practice, however, it should not cause a lack of color uniformity in a commercial product. Considering the above relationships and other effects of heat upon color, chiefly that of time of forewarming, and of the effect of heat upon concentration, certain conclusions seem justified. The darkening of color caused by heat in evaporated milk seems to be of a catalytic nature, dependent probably upon the production of substances in the solution which increase the susceptibility of the lactose to caramelization.

The only important neutralizer which appears to affect appreciably the color of the product is sodium bicarbonate. The increase of color due to the addition of this salt was not found to be uniform for all milks to which it was added. Where no change in pH was brought about by addition of the neutralizer, as when salt was added after it had been neutralized with lactic acid, no darkening of the color of the finished product was observed. In those cases in which color darkening was observed, the addition of the neutralizer did not bring about maximum stability in the darkest colored samples. It appears, therefore, that where neutralization is properly conducted to obtain maximum stability, minimum darkening of color will result, but where overneutralization is practiced the color will be unduly increased. In no case, however, will the color of neutralized milk be as light as that of the milk to which no salt has been added.

The complete color change of an evaporated milk as presented

in figure 8 shows it to be of a catalytic nature during steriliza-
tion, while the change during storage, which is proportional to
time and temperature, is one of chroma only, giving the milk a
yellowish-green color. The fact that the color change during
storage does not continue along the sterilization curve seems to
indicate that factors other than caramelization of the lactose
affect the color of the stored milk.

SUMMARY

Permanent color standards have been developed, expressed
in numerical terms according to the Munsell system.

Color changes caused by variation in the manufacturing process
of evaporated milk have been studied. The effect of heat,
whether encountered during forewarming, sterilization, or stor-
age, was found to produce important changes in color. An in-
crease in heat produced an increase in color, the change being
of a catalytic nature. Increase in heat during sterilization
deepened color by affecting brilliance, chroma, and hue; while
increase in time and temperature of storage deepened color
through increase in chroma only. The use of sodium bicarbonate
as a neutralizer darkened the color of evaporated milk in propor-
tion to the amount used.

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A COMPARISON OF THE VOLUMETRIC AND GRAVI-METRIC METHODS FOR BACTERIOLOGICAL EXAMINATION OF ICE CREAM*

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INTRODUCTION

A bacteriological analysis of ice cream presents certain difficulties which hinder the procurement of a fair sample. Obviously, the air incorporated in the product must be completely expelled if uniform results from volumetric (1- or 10-cc.) samples are to be expected. On the other hand, if the analysis is made on a gravimetric basis (10-gram), the sample need only be melted sufficiently to enable thorough shaking. Variation in the viscosity of different ice cream mixes and the use of glassware that is not chemically clean results in more or less of the mix adhering to the pipette. With a volumetric sample this may offer a source of considerable variation, whereas with a gravimetric portion it does not matter how much mix adheres to the pipette or how much air is retained in the product. There has been some question in the minds of those who make bacterial determinations on ice cream as to whether the advantages of the gravimetric sample justify the extra time and work involved. The use of the gravimetric method necessitates careful counter-balancing of the dilution blank, followed by carefully weighing 10 grams of the melted ice cream. The two operations with the balances are both time consuming and tedious. Bolling and his associates (4) found that the gravimetric method required 77 per cent more time than the volumetric procedure and concluded that, "equally accurate counts could be obtained by either method." The extra labor involved in the gravimetric method would be justified only if the results were less variable than would be obtain-

able from the more simple and less time consuming volumetric method. The following experiments were designed primarily to ascertain the comparative degree of variability of the gravimetric and volumetric methods.

PLAN OF THE EXPERIMENT

After preliminary plating of a sample of ice cream to determine the desired dilution, about 250 cc. of the frozen product were

TABLE 1

A comparison of the results obtained with gravimetric and volumetric samples in the bacteriological analysis of ice cream

EXPERIMENT NUMBER	DILUTION	NUMBER OF PLATES	AVERAGE NUMBER OF COLONIES PER PLATE			PROBABLE ERROR OF SINGLE PLATES EXPRESSED IN COLONIES PER PLATE			NUMBER OF PLATES NEEDED TO INSURE (30 TO 1 CHANCE) THAT THE DIFFERENCE BETWEEN THE MEANS IS SIGNIFICANT—COMPARISON OF					
									10-gram and		10-cc and		1-cc. and	
			10-gram sample	10-cc. sample	1-cc. sample	10-gram sample	10-cc. sample	1-cc sample	10-cc sample	1-cc sample	10-gram sample	1-cc sample	10-gram sample	10-cc. sample
1	1:1,000	150	32	33	36	4 65	4.99	4 78	221	14	255	28	15	26
2	1:10,000	148	52	54	56	4 71	5.43	5.90	57	14	75	75	22	89
3	1:100	144	150	157	130	25 48	19.78	33 51	135	17	82	5	29	16
4	1:1,000	118	169	192	180	17.76	16.69	23 85	6	27	5	20	48	40
5	1:500	150	194	180	187	28.23	12.70	14 47	42	166	8	34	44	44
6	1:100	150	226	231	248	20.26	22.62	24.67	168	9	209	18	13	22
7	1:10,000	149	269	231	205	45 15	26 04	36 05	14	5	5	10	3	20
8	1:1,000	150	227	266	305	29 05	38.91	23 47	71	11	128	10	7	4
9	1:1,000	150	465	538	566	101.42	70.41	74.76	20	10	10	65	6	73
10	1:1,000	149	2,270	2,175	2,339	185.59	184.81	205 85	39	74	39	13	91	16
11	1:500	150	120	124	146	27 00	24 63	32 15	467	11	388	13	16	22
Average....			384	310	400				112	32	109	26	26	33

melted in a water bath at 45°C. until as much of the air had been expelled as possible. The time required for melting did not exceed fifteen minutes in any case.

Five sets of dilutions were made from 10-gram samples and 10 plates were poured from the final dilution of each set, thereby making 50 plates on a gravimetric basis. Similarly, 5 sets of dilutions each were prepared from 10-cc. and 1-cc. volumetric samples

and 50 plates poured from the final dilutions in each case. In no case did the elapsed time between weighing or measuring the sample and pouring of the agar exceed fifteen minutes. Occasionally it was necessary to eliminate a contaminated plate, and in a few instances to eliminate a whole series of 10 plates, due to a contaminated dilution blank. In most instances, however, the data are based on the entire set of 150 plates.

The experiment as described was followed on 10 samples of ice cream. Another procedure which was followed for an eleventh sample will be described when the data are presented.

The methods used for preparation of media, sampling, weighing, plating, and incubation were those set forth in the report of the American Dairy Science Committee on Bacteriological Methods for Examining Ice Cream (1).

In selecting the samples of ice cream for analysis and in estimating the desired dilution, an attempt was made to obtain sets of 150 plates with numbers of colonies ranging from a few colonies per plate (about 30) to an excessive number.

By statistical treatment of the data collected, it is possible to show the comparative variation of the three methods of sampling. It is also possible to determine whether or not the differences in the results obtained by the three methods are significant.

RESULTS

A study of the average counts reported in the table shows that in some experiments the 1 cc. volumetric method of sampling yielded the highest average count, whereas in other samples one of the other methods gave the highest counts. Space does not permit a detailed discussion of the figures in this table, but only a brief examination of the data will convince the reader that neither method consistently gave the highest or lowest results.

Mudge and Lawler (2) have questioned the applicability of statistical methods to plate counting. Their criticism is based on the observation of factors other than chance which affect the results when a large number of plates are prepared from a single dilution blank. Their results show that in making 75 plates from the same dilution blank, the last 35 of the series give mate-

rially higher counts than the first 40 plates, even though the dilution blanks are chilled.   More recently (3) these investigators have attributed this phenomenon to the lack of buffering effect in distilled water dilution blanks, and have found that it could be prevented by using buffered solutions for dilution.

Obviously, an erroneous conception of the degree of variability of the plate count would be drawn if data included in the computations are affected by this phenomenon.   Although the work reported in the present paper was completed before Mudge and Lawler's report of the use of buffered dilution blanks, an effort was made in these experiments to eliminate the error introduced by this phenomenon.

The results obtained by Mudge and Lawler indicate that some force is at work in the dilution blank which causes the bacterial clumps to disintegrate.   Whatever the cause of this phenomenon may be, its appearance is more or less sharply defined after about 40 plates have been poured.   It is not known whether the clumps suddenly disintegrate after about 40 plates have been poured or whether the process begins immediately after making the dilution and only attains significant magnitude after a given time interval. If the former be the case, statistical analysis of the results of more than about 40 plates per dilution blank would be unjustified and misleading.   On the other hand, if the separation of clumps in the unbuffered blanks is a gradual process, it is one of the factors of normal variation of the plate method up to fifteen minutes after the preparation of the dilution, and should be included in a statistical analysis.   In either event, it is evident from the work of Mudge and Lawler, that a statistical analysis of plate counting should not be based on more than 30 to 35 plates from single unbuffered dilution blanks.   On the other hand, unless buffered dilution blanks were commonly employed in laboratory procedure, it would be equally misleading to judge the error of plate counting from data based on series of 75 or 100 plates made from buffered dilutions.

In view of the facts that the unbuffered blanks, as employed in this work, are commonly used for dilution, that only 10 plates were poured from each blank, and that the time interval between

making and plating the dilution did not exceed fifteen minutes, it is believed that the variations in these data are of practical significance. The statistical constants are likewise believed at least to be representative of the expected limits of variation which will be encountered in bacterial analyses by the plate method. The statistical values given are not directly applicable to the work of another laboratory and, very likely, not applicable to previous or subsequent work of this laboratory. At best, these values give only some tangible evidence of the general range of variation of the plate method of analysis.

In the first experiment, it may be observed that the probable error of single plates made from the 1-cc. samples was the lowest; in the second experiment, the 10-gram samples gave the least variation; and in the third experiment, the 10-cc. samples showed less variation than the others. If the remainder of the figures in these three columns are examined it will be observed that neither method of sampling is significantly superior to the others. With one sample of ice cream one method may yield slightly less varying results, but with the next sample the degree of variation previously observed may be completely reversed.

In order to further illustrate that there is little on which to base a choice of these methods of sampling, in the last six columns of the table are presented the number of plates required to show a significant difference in the results obtained by various combinations of methods. The figures for experiment 5 will be used for explanation. It will be noted that the averages of 50 plates each from 10-gram, 10-cc. and 1-cc. samples were 194, 180, and 187 colonies respectively. Since these values were obtained from averaging a larger number of plates than would ever be made in routine analysis, it will be arbitrarily assumed, for sake of the subsequent explanation, that for this ice cream 194, 180 and 187 are the "true values" for these respective methods of sampling. On the basis of this assumption, therefore, the difference of 14 colonies (194 − 180) represents the difference in the counts obtained with 10-gram, and 10-cc. samples, and, likewise, 7 colonies represents the difference when the 10-gram and 1-cc., and the 10-cc. and 1-cc. samples are compared. The figures in the table

indicate that in order to demonstrate conclusively (30 to 1 chance) that the difference between the 10-gram and 10-cc. samples is real, it would require 42 plates by the gravimetric method and 8 plates by the 10-cc. volumetric method. Simliarly, in order to demonstrate a significant difference between results obtained with the 10-gram and 1-cc. volumetric methods it would require 166 plates by the former and 44 by the latter. Likewise, it may be seen that it would take 34 plates by the 10-cc. volumetric method and 44 by the 1-cc. method to demonstrate the significance of the difference in the results of these two methods of sampling.

In determining the number of plates required to demonstrate the significance of the difference between the averages of 50 plates the following formula was used,

$$N = \left(\frac{c \cdot p}{d} \right)^2,$$

where N = number of plates

c = coefficient of odds (3.2 used in this case which gives 30 to 1 chance)

p = probable error of a single plate

d = difference between the means

When comparing two methods, e.g. the 10-gram and 10-cc. samples, it was necessary to calculate the requisite number of plates for each method of sampling since the degree of variation (p) was not the same in each case.

A study of this table indicates that in order to demonstrate a preference of one method of sampling over another it would require far more plates than are ordinarily used in routine analytical work. The required number varies from 3 to 467 plates, and in only 11 of the 66 comparisons was the number of plates less than 10.

As previously stated, a different procedure was followed in the analysis of the eleventh sample of ice cream (experiment 11). In this experiment 25 separate samples from the same can of ice cream were taken in 25 sterile sample jars. All of the samples were melted simultaneously in the same water bath and for the same length of time. After melting all were placed in iced-water until used. From each sample of the melted ice cream a 10-gram,

a 10-cc., and a 1-cc. portion were taken for subsequent dilution. From the final dilution duplicate plates only were poured. Thus it may be seen that there were 25 separate weighings of 10-gram portions from 25 separate sample jars of the same ice cream, and that there were duplicate plates for each determination. Similarly, there were 25 separate determinations (50 plates) each on a 10-cc. and a 1-cc. basis. It was thought that in some respects the results from this procedure would more closely approximate the error of the method as used in practice than the procedure followed in the other experiments.

It may be noted in experiment 11 that the plates from the gravimetric samples averaged 120 colonies per plate with a probable error for a single plate of 27 colonies; the 10-cc. volumetric samples averaged 124 colonies per plate with a probable error for a single plate of 24.63 colonies; and the plates from the 1-cc. samples averaged 146 colonies with a probable error for a single plate of 32.15 colonies. The number of plates necessary to show a preference of one method over another varied from 11 to 467, again emphasizing that the results of routine analysis in which duplicate or triplicate plates are used would not yield significantly different results regardless of the method employed.

DISCUSSION

It is impractical to make fine measurements with a crude measuring stick. The error inherent in the plate method obliterates the slight advantage which one type of sample may have over another. It undoubtedly is true that a 10-cc. sample is more representative than a 1-cc. sample. Nevertheless, in these experiments, the counting of approximately 500 plates by each of the methods indicates that the differences in results obtained are so small as to require large numbers of plates to substantiate a preference. Similarly, any theoretical advantage which may accrue from the use of a 10-gram gravimetric sample necessitates in most instances excessive numbers of plates to corroborate. The data indicate quite clearly that the errors introduced by the various methods of sampling are overshadowed by the error of the plating method.

After studying these results one is forced to conclude that it makes little difference whether an ice cream analysis is based on a 10-gram, 10-cc. or 1-cc. sample. Although it is perfectly logical to assume that a 10-gram sample eliminates certain errors, and that a 10-cc. sample is more representative than a 1-cc. sample, nevertheless, the normal variation tends to obliterate the superiority which any one method may possess. If, as indicated in these data, either of the volumetric samples yields results as reliable as the more tedious and time consuming gravimetric procedure, there is little to justify the use of the latter method. On the other hand, a 10-gram sample certainly would be advisable when an occasional sample of ice cream is found to be frothy on melting and does not give up its incorporated air. In choosing between a 10-cc. and a 1-cc. sample, it seems logical to recommend the 10-cc. sample because it is more representative, however, it would be difficult to substantiate the preference if the plate method of analysis were employed.

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SOME FACTORS INFLUENCING THE VOLUME OF FOAM ON MILK*

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The appearance of foam on milk and some of its products is a commonly observed phenomenon. In some instances, as in the whipping of cream or in the manufacture of ice cream, a thick, stable foam is desired. In other instances, as in the filling of vats, cans, bottles, or other containers, or in the operation of machines, such as clarifiers, separators, pasteurizers, etc., the formation of foam may present a serious technical problem. The overflow of foam when vessels are to be filled may result in the loss of a considerable quantity of milk solids.

It is known to bacteriologists that in the commercial pasteurization of milk, the destruction of bacteria in the foam layer is far less complete than in the remainder of the milk. Whittaker, Archibald, Leete, and Miller (9) have shown that the temperature of the foam on the surface of a vat of milk during the pasteurization process may be much below that which is necessary to insure complete destruction of the pathogenic bacteria sometimes found in milk. They have shown, also, that under certain conditions which are frequently found in dairy plants, the bacterial content of the foam layer may actually increase during pasteurization.

Rahn (3) claims that milk contains a special foam compound (a protein), which is neither casein nor albumin, but which probably is the protein that surrounds the fat globules.

Measurements reported by Siedel (8) indicate that whole milk churned for about forty-five minutes at a low temperature has lost its ability to produce foam, on the skim milk obtained from it subsequently by centrifugal separation. This author also

reported data showing that the loss of foaming ability was not due to the low temperature during the churning but rather to the mechanical agitation of the milk.

The associates of Rogers (7) report that the tendency for milk to foam is at a minimum between the temperatures of 20° and 30°C. (68° and 86°F.). Below this range the foaming tendency increases with decreases in temperature; above this range, there is a rapid increase in the foaming tendency with increases in temperature. These authors also report that pasteurization slightly reduces the tendency to foam at the various temperatures, and that the region of minimum foaming ability shifts to a higher temperature as the fat content of the milk increases.

Dahlberg and Hening (1) report an experiment in which a sample of pasteurized skim milk whipped at 4.4°C. attained a much larger volume than a similar sample of raw skim milk. Milk with 10 per cent of fat whipped to a much smaller volume than did skim milk.

Larson (2) in a preliminary report stated that foam production on milk took place in a vacuum to approximately the same degree as under atmospheric pressure.

The present investigation was planned to make a systematic study of the influence of certain factors on the foaming ability of cow's milk.

METHOD

Portions of the sample, each 250 ml. in amount, were measured with a graduated cylinder and transferred to 500-ml. graduated beakers. The different portions were tempered in the beakers in baths of water to the temperatures required. Each measured sample was then whipped for one minute in the beaker by an electrical stirring machine, such as is used commonly at soda fountains in the preparation of malted milks and other drinks. At the end of the minute the agitator was quickly withdrawn. Fifteen seconds was allowed for the surface of the foam to come to rest, after which the total apparent volume of the sample (including foam) was read quickly.

The apparent increase in volume of the sample was calculated

as a percentage of the original volume (250 ml.) and designated as the "percentage increase in volume due to foam."

Preliminary trials indicated that duplicate observations on the same sample could be expected to fall within ±5 ml. of the mean when small apparent increases in volume occurred, and within ±10 ml. of the mean when the larger increases were encountered. Usually duplicates agreed closer than these extremes. In some cases it was necessary to calibrate the beakers beyond the 500-ml. mark.

TABLE 1

Effect of temperature at time of whipping on foaming ability

TEMPERATURE	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM		
	Whole milk	Skim milk	20 per cent cream
°F.			
35	88	130	49
40	73	122	40
50	30	84	36
60	11	28	26
70	8	18	20
80	8	17	15
90	16	21	8
100	29	26	31
110	32	29	35
120	31	28	37
130	30	27	37
140	28	25	35
150	26	24	31
160	25	24	29
170	23	23	27
180	22	21	25

EFFECT OF TEMPERATURE ON FOAMING ABILITY

Temperature of the sample at the time of whipping

The foaming ability of each of 8 lots of commercially pasteurized milk, 6 lots of commercially pasteurized skim milk, and 2 lots of commercially pasteurized 20 per cent cream was determined at temperatures from 35° to 180°F. The averages of the results are shown in table 1.

The data indicate that in all 3 products, the greatest amount of

foam was produced at the lowest temperature of measurement. With increases in temperature, the volume of foam decreased until a minimum was reached. This minimum was reached at 70° to 80°F. with whole milk, 80°F. with skim milk, and 90°F. with 20 per cent cream.

Further increases in the temperature of the samples resulted in increasing the foaming ability, until a second maximum point was reached. This second maximum point was reached at 110°F. with whole milk and skim milk, and at 120° to 130°F. with 20 per cent cream.

The results show, also, that at low temperatures skim milk foamed most, whole milk next, and 20 per cent cream least. At the higher temperatures of measurement, the reverse relationship existed. If the data were plotted, it would appear from the graphs that at about 96° to 97°F. the volumes of foam on the 3 products should be almost identical.

Previous heating (pasteurization)

Separate portions of the same lots of fresh whole milk (4 lots) and of fresh skim milk (3 lots) were heated for thirty minutes at 140°F. Separate portions of other lots of fresh whole milk (3 lots) and of fresh skim milk (3 lots) were heated for thirty minutes at 180°F. The heated portions were promptly cooled to below 50°F., and distilled water added to replace that lost by evaporation. All of the samples, together with unheated controls, were stored for two or three days in a refrigerator at 40°F.

Measurements of the foaming ability were then made in duplicate, at temperatures of 40°, 80°, 140°, and 180°F. The averages of the results are shown in table 2.

The results indicate that heating milk or skim milk for thirty minutes at 140°F. had no significant effect on the foaming ability. When the heating was done at 180°F. for thirty minutes, there was a slight but regular decrease in the foaming ability.

Freezing

Samples of pasteurized whole milk, skim milk, and 20 per cent cream were kept at a temperature of about −10°F. for two days.

The frozen samples then were kept at room temperature for a day to melt, and thereafter stored for two or three days at about 40°F. Control samples were kept continuously at 40°F.

Duplicate measurements of the foaming ability were made at 40°, 80°, and 140°F. The results are shown in table 3.

TABLE 2

Effect of previous heating on foaming ability

DESCRIPTION OF SAMPLE	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM			
	40°F.	80°F.	140°F.	180°F.
Raw whole milk, control (4 lots)........	94	7	26	20
Same, but heated, 140°F.—30 minutes...	89	8	27	20
Raw skim milk, control (3 lots)	133	26	28	27
Same, but heated, 140°F.—30 minutes...	133	28	27	23
Raw whole milk, control (3 lots).	95	7	27	*
Same, but heated, 180°F.—30 minutes.	78	6	25	*
Raw skim milk, control (3 lots)........	117	27	29	*
Same, but heated, 180°F.—30 minutes...	112	22	26	*

* Measurements omitted.

TABLE 3

Effect of freezing on foaming ability

DESCRIPTION OF SAMPLE	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM		
	40°F.	80°F.	140°F.
Whole milk, control........	95	8	27
Whole milk, frozen....	89	7	27
Skim milk, control..............	136	31	29
Skim milk, frozen.....	136	30	30
20 per cent cream, control......	54	11	38
20 per cent cream, frozen...... .	38	16	38 ("oiled off")

The results indicate that freezing decreases slightly the foaming ability of whole milk when the measurements are made at 40°F., but not at 80° or 140°F. The results with 20 per cent cream indicate a considerable decrease when the measurements are made at 40°F., an increase at 80°F., and no change at 140°F. There appears to be no effect on the foaming ability of skim milk as the result of freezing.

It seems that the effect of freezing on the foaming ability may be due to the physical condition of the milk fat. Reid (6) has shown that in freezing the globules of fat in milk become much distorted and grouped. Apparently, the distorted and grouped globules interfere mechanically with the normal tendency of the milk to foam. When the measurements were made at 80° or 140°F., at which temperatures the fat globules were softened or melted, the decrease in foaming ability was not noted. Skim milk, containing but a trace of fat, showed no change due to freezing.

EFFECT OF AGE AND OF STORAGE TEMPERATURE ON FOAMING ABILITY

In a preliminary experiment with 6 lots of whole milk from various sources, it was found that when the milk was passed through a centrifugal separator while quite fresh, the foaming ability of its skim milk was much greater than after the milk had been held for from twenty to seventy-two hours in a refrigerator. The results suggested that the foaming ability of milk might be expected to decrease with age.

Milk was obtained at milking time and poured into a warmed insulated can. One portion was at once poured into warmed milk bottles and stored at 98°F. for three to four hours. Another portion was cooled quickly in ice water to 40°F., and stored at that temperature for three to four hours. The foaming ability of the original, fresh milk was determined as a control. Duplicate measurements were made at 40°, 80°, and 140°F. The foregoing operations were completed within an hour after the collection of the milk at the barn was begun.

The foaming ability of the milk stored at 98°F. and of that stored at 40°F. were determined as above. The experiment was repeated with a second lot of milk. The averages of the results are shown in table 4.

These results indicate that the temperature at which milk is held is significant in determining the effects of age on the foaming ability of the milk. The measurements made after the milk had been held for three to four hours at 98°F. were practically identical with those made when the milk was freshly drawn. In

the freshly drawn milk, and in the milk held at 98°F., the measurements made at 80°F. and at 140°F. were approximately the same. In the preceding experiments in this study (all with previously cooled milk), the foaming ability of whole milk at 80°F. was found to be much less than at 140°F.

The milk which had been held at 40°F. for three to four hours showed a slight decrease in its foaming ability as determined at 40°F., and a great decrease as determined at 80°F. All of the samples showed the same foaming ability when the measurements were made at 140°F.

Apparently, the solidification and clumping of the fat globules, which occurs when milk is held cold, influences the foaming ability of the milk. In the sample stored cold and then tempered to

TABLE 4

Effect of age and of storage temperature on foaming ability

TREATMENT OF SAMPLE	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM		
	40°F.	80°F.	140°F.
Fresh, control...........................	98	24	25
At 98°F. for 3–4 hours..................	100	26	25
At 40°F. for 3–4 hours....	86	8	25

80°F. the fat globules continued in the solid state, but certainly became somewhat softened. In the freshly drawn sample, and in the sample held at 98°F. when tempered to 80°F., the fat globules undoubtedly continued practically in the liquid state. In all of the samples, when tempered to 140°F., the fat globules were in the liquid condition.

EFFECT OF PREVIOUS AGITATION ON FOAMING ABILITY

Four different lots of raw whole milk, 3 of skim milk, and 2 of 20 per cent cream were each divided into 2 portions. About 3 or 4 gallons of one portion in each case was whipped for one hour in a motor driven 50-quart ice cream freezer cooled by brine. The temperature of the sample in the machine was kept between freezing and 40°F.

The second portion in each case was heated to 145°F., and distilled water added to replace that lost by evaporation. About 3 or 4 gallons of the heated sample in each case was whipped for one hour in the ice cream freezer. The machine had been heated previously so that the temperature of the product remained above 120°F. during the whipping. A sample of the heated, unwhipped portion was kept at practically the same temperature as long as the whipping continued. After the whipping, all of the heated samples were cooled promptly in ice water.

TABLE 5

Effect of previous agitation on foaming ability

| TREATMENT OF SAMPLE | PERCENTAGE INCREASE IN VOLUME DUE TO FOAM | | | | | | | | |
| | Whole milk | | | Skim milk | | | 20 per cent cream | | |
	40°F.	80°F.	140°F.	40°F.	80°F.	140°F.	40°F.	80°F.	140°F.
Control (a)	87	7	23	136	19	28	44	11	33
Whipped at 40°F. or under (b)	31	4	15	128	14	23	*	*	*
Heated to 120°F. or over (c)	92	8	24	137	18	28	42	11	32
Whipped at 120°F. or over (d)	128	16	24	140	30	30	65	12	28
Decrease due to whipping cold (a–b)	56	3	8	8	5	5	—	—	—
Decrease due to heating and whipping warm (a–d)	−41	−9	−1	−4	−11	−2	−21	−1	5
Decrease due alone to whipping warm (c–d)	−36	−8	0	−3	−12	−2	−23	−1	4

* Badly churned; no measurements.

All of the samples and controls were stored for a day in a refrigerator at 40°F. Measurements of the foaming ability were made in duplicate at 40°, 80°, and 140°F. The averages of the results are shown in table 5.

The results indicate that the foaming ability of whole milk or skim milk was diminished by agitation while cold. When whole milk, skim milk, and 20 per cent cream were whipped at a temperature well above the melting point of milk fat, and subsequently cooled, the foaming ability generally was increased (negatively decreased).

Rahn (3) and Rahn and Sharp (4), citing Siedel's (8) data, claim that the formation of foam is an irreversible process. They claim that the foam substance solidifies in the surface film (foam) according to the phenomenon described by Ramsden (5), and then is no longer capable of producing foam. Previous agitation, which produces foam, then should decrease the subsequent foaming ability of the milk. The results obtained with the lots which were agitated while cold harmonize with this view. When the products were agitated while warm the data obtained gave no evidence that the foam substance was inactivated.

TABLE 6

Effect of homogenization on foaming ability

TEMPERATURE OF MEASUREMENT	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM			
	Control heated only	Homogenized at 3000 pounds— first valve only	2000 pounds, first valve; 1000 pounds, second valve	3000 pounds, first valve; 1000 pounds, second valve
Whole milk				
°F.				
40	91	112	112	109
80	7	20	18	19
140	29	24	25	23
Skim milk				
40	131	136	137	134
80	29	33	34	33
140	28	31	31	32

EFFECT OF HOMOGENIZATION ON FOAMING ABILITY

A lot each of raw whole milk and skim milk were heated to 145°F. A portion of each heated lot was kept as a control. Other portions of each were passed through a two-stage homogenizer, such as is used in commercial dairy plants. Various degrees of pressure were used, as indicated in the table. The various homogenized portions and the controls were cooled promptly in ice water and then stored overnight in a refrigerator at 40°F. After storage, the foaming ability of each was determined in duplicate at 40°, 80°, and 140°F. The results are shown in table 6.

The data indicate that homogenization increased considerably the foaming ability of whole milk as measured at 40° and 80°F. There was a decrease when the measurements were made at 140°F. In all cases, the homogenized skim milk showed a slightly increased foaming ability.

EFFECT OF COMPOSITION ON FOAMING ABILITY

Two lots of milk from individual Ayrshire cows and 2 from individual Holstein cows were obtained. All were analyzed for fat and total solids. The foaming ability of each lot was determined, after cooling and storing overnight in a refrigerator. The measurements were made in duplicate at 40°, 80°, and 140°F. The results are shown in table 7.

TABLE 7

Effect of composition on foaming ability of milk from individual cows

SOURCE OF SAMPLE	FAT	TOTAL SOLIDS	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM		
			40°F.	80°F.	140°F.
	per cent	per cent			
Ayrshire Cow 1........	4 6	14 28	85	8	28
Ayrshire Cow 2	5.2	14 88	94	15	33
Holstein Cow 1.	3 4	13 09	98	11	28
Holstein Cow 2	3 7	12 20	125	9	25

The results fail to indicate any definite relationship between the fat and total solids content of milk and its foaming ability. Apparently, the foaming ability of milk from individual cows is dependent largely on factors which are characteristic for the animals.

To determine the effect of composition on the foaming ability when the influence of the individual characteristics of the milk was standardized, samples were prepared by mixing high testing cream, skim milk, and distilled water in varying proportions. The cream and skim milk were obtained by machine separation of high testing milk produced by a herd of Jersey and Guernsey cows. The cream and skim milk were cooled and at once analyzed for their content of fat and solids not fat. As promptly

as possible, the cream, skim milk, and distilled water were mixed
in separate lots to yield the possible combinations of 0, 2, 4, or
6 per cent of fat, with 1, 5, or 9 per cent of solids not fat.   The
prepared samples were stored overnight in a refrigerator at 40°F.,

TABLE 8

Effect of composition on the foaming ability of various prepared samples

Part A

	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM								
FAT	1 per cent solids not fat			5 per cent solids not fat			9 per cent solids not fat		
	40°F.	80°F.	140°F.	40°F.	80°F.	140°F.	40°F.	80°F.	140°F.
per cent									
0	59	26	14	138	26	21	136	29	28
2	25	4	9	100	7	19	115	10	27
4	19	4	11	76	6	23	99	8	27
6	16	5	10	66	5	22	88	7	31

Part B

COMPOSITION OF MIXTURE			PERCENTAGE INCREASE IN VOLUME DUE TO FOAM		
Total solids	Fat	Solids not fat	40°F.	80°F.	140°F.
per cent	*per cent*	*per cent*			
5	0	5	138	26	21
5	4	1	19	4	11
7	2	5	100	7	19
7	6	1	16	5	10
9	0	9	136	29	28
9	4	5	76	6	23
11	2	9	115	10	27
11	6	5	66	5	22

and the foaming ability of each determined.   The measurements
were made in duplicate at 40°, 80°, and 140°F.

The experiment was performed 3 times, using different original
lots of milk.   The averages of the results are shown in table 8.

The data indicate that when the influence of the individual
characteristics of the milk was standardized, the fat generally

had a depressing influence on the foaming ability of the sample, while the solids not fat generally increased the foaming ability. This is best shown in Part B of the table.   In each pair of samples, the one with the more fat had the lesser foaming ability.

EFFECT OF DEVELOPED ACIDITY ON FOAMING ABILITY

Different lots of sweet whole milk and skim milk were tempered to 72°F. and inoculated with small amounts of lactic dairy starter. The lots were each divided into several portions, which were incubated for varying lengths of time at room temperature.

TABLE 9

Effect of developed acidity on foaming ability

Whole milk

PER-CENT-AGE ACIDITY	PERCENTAGE INCREASE IN VOLUME DUE TO FOAM											
	0.16	0.18	0.19	0.20	0 27	0 31	0.38	0 46	0 58	0.71	0.76	0.80
°F.												
40	104	104	102	104	109	119	134	135	130	103	63	42
80	9	8	8	8	9	8	8	9	9	10	10	12
140	29	29	28	28	27*	25*	24*	†	†	†	†	†

Skim milk

	0.15	0 17	0.19	0.22	0.30	0.33	0.44	0.57	0.68	0.80	0.83
40	133	134	136	134	134	135	135	147	138	87	57
80	20	20	19	20	20	20	21	20	20	20	29
140	29	28	28	28	27	27	19*	†	†	†	†

* Slight curdling during tempering.

† Curd separated; no measurements.

They were then stored for about two days in a refrigerator at 40°F.

The foaming ability in each case was determined after storage, duplicate measurements being made at 40°, 80°, and 140°F.   The titratable acidity, calculated as lactic acid, was determined at the same time.   The experiment was performed with 4 different lots each of whole milk and skim milk.   The results with a representative lot of each product are shown in table 9.

These results indicate that moderate amounts of developed acidity increased the foaming ability of milk, when the measure-

ments were made at 40°F. Further increases in developed acidity reduced the foaming ability. The decrease apparently is associated with the precipitation of the curd in the milk. When the measurements were made at 80°F., there were no significant variations until considerable amounts of acid had developed; a slight increase was noted then. The measurements made at 140°F. showed no significant variations due to acidity, until enough was present to bring about the formation of a slight curd during the tempering process.

The results with skim milk in general were comparable to those with whole milk.

EFFECT OF CERTAIN ADDED SALTS ON FOAMING ABILITY

Various mixtures were prepared from whole milk, distilled water, and m/2 solutions of each of 5 different salts, as indicated in the table. The mixtures, together with control samples of milk, were stored for a day at about 40°F. The foaming ability of each was determined after storage. The measurements were made in duplicate at 40°, 80°, and 140°F. The results are shown in table 10.

When the measurements were made at 40°F. added calcium lactate reduced the foaming ability of the milk, and added primary magnesium phosphate caused an increase. No significant changes in the foaming ability could be attributed to any other of the salts added, when the measurements were made at 40°F. When the measurements were made at 80°F. no significant changes could be attributed to any of the salts added. When the measurements were made at 140°F. the addition of either calcium lactate or primary magnesium phosphate brought about a decrease in the foaming ability. A partial curdling appeared in these samples when they were tempered to 140°F. The formation of the curd was accompanied by a decrease in the foaming ability.

Sodium citrate, when added in the larger amounts increased slightly the foaming ability of the milk measured at 140°F. Under the conditions of the experiment, added sodium bicarbonate or disodium phosphate showed no effect on the foaming ability of the milk, at any of the temperatures of measurements.

TABLE 10

Effect of various added salts on foaming ability

COMPOSITION OF MIXTURE			PERCENTAGE INCREASE IN VOLUME DUE TO FOAM		
Milk	M/2 SALT SOLUTION	Water	40°F.	80°F.	140°F.
Calcium lactate					
ml.	*ml.*	*ml.*			
Control	0	0	102	8	27
1900	0	100	101	6	28
1900	40	60	92	6	26*
1900	60	40	80	6	24*
1900	100	0	77	7	23*
Primary magnesium phosphate					
Control	0	0	98	6	7
1900	0	100	98	6	26
1900	40	60	101	6	24*
1900	60	40	105	6	21*
1900	100	0	117	6	21*
Sodium bicarbonate					
Control	0	0	95	8	27
1900	0	100	94	8	27
1900	40	60	95	7	27
1900	60	40	95	8	27
1900	100	0	96	7	27
Sodium citrate					
Control	0	0	102	8	27
1900	0	100	101	6	28
1900	40	60	103	8	29
1900	60	40	104	7	34
1900	100	0	102	8	36
Disodium phosphate					
Control	0	0	95	8	27
1900	0	100	94	8	27
1900	40	60	95	8	26
1900	60	40	94	8	29
1900	100	0	95	7	28

* Slight curdling during tempering.

SUMMARY AND CONCLUSIONS

A number of the factors influencing the volume of foam appearing on milk were studied. As a measure of the foaming ability of a sample, the "percentage increase in volume due to foam" was determined. The foaming ability of milk varies widely under different conditions. It is necessary, therefore, to specify the conditions under which the measurements are made.

Low temperatures favor the production of the largest volume of foam. With increasing temperature, the volume of foam decreases to a minimum, then rises again with increasing temperature to a second maximum point, lower than the first maximum; this is followed by a decrease in the volume of foam as the temperature rises.

Freshly drawn milk does not exhibit so low a minimum foaming ability as previously cooled and aged milk. The effect of age on the foaming ability of milk seems to be dependent in part on the changes taking place in the fat globules during the aging period. These changes, in turn, are partly dependent on the temperature at which the milk is held.

Agitation of milk at a low temperature reduces its subsequent ability to foam. Agitation at a temperature which is high enough to cause the fat globules to be in a liquid condition increases the subsequent foaming ability of the milk.

There seems to be no definite relationship between the composition and the foaming ability of milk from individual cows. When the influence of the individual characteristics is standardized, increases in the fat content generally decrease the foaming ability of milk, and increases in the milk solids not fat content generally increase the foaming ability.

Moderate amounts of developed acidity increase the foaming ability of milk, as measured at 40°F.; when there is enough developed acidity to cause a curdling of the milk in the cold, the foaming ability decreases. When the measurements are made at 80°F., no significant changes in the foaming ability appear until there is a considerable amount of developed acidity; then a slight increase occurs. When the measurements are made at 140°F., a decrease in the foaming ability is evident as soon as there is

enough developed acidity to bring about a slight curdling of the milk during the tempering process.

When the measurements are made at 40°F., added calcium lactate decreases the foaming ability of milk; added primary magnesium phosphate increases the foaming ability. When the measurements are made at 80°F., none of the added salts as used in this study bring about any change in the foaming ability of milk. When the measurements are made at 140°F., added calcium lactate and primary magnesium phosphate each cause a decrease in the foaming ability; this decrease accompanies a slight curdling of the milk during the tempering process. Added sodium citrate causes a slight decrease in the foaming ability of milk, when the measurements are made at 140°F. Added sodium bicarbonate or disodium phosphate as used in this study have no effect on the foaming ability of milk.

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VARIATIONS IN STREPTOCOCCUS LACTIS*

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The action of *Streptococcus lactis* is of a great deal of importance in the field of dairying because of the frequency with which this species grows in dairy products. Variations in the *S. lactis* organisms are rather common and in milk or cream fermented by them differences in flavor and aroma, consistency, rate of coagulation, etc., are encountered. Many of these differences, although of little importance to the systematic bacteriologist, are very significant in the dairy industry.

It appears that *S. lactis* cultures showing a definite difference in a certain character may sometimes be secured from what would ordinarily be regarded as a pure culture. The work herein reported was done in an attempt to secure, from a pure culture, organisms differing in their response to air supply; it was suggested by the observation that *S. lactis* cultures from a certain source regularly began their development at the tops instead of the bottoms of tubes of litmus milk.

HISTORY OF THE CULTURES STUDIED

One of the extensively used butter cultures contains lactic acid producing organisms that differ from the lactic acid organisms of most butter cultures by beginning the reduction and coagulation of tubes of litmus milk at the tops instead of the bottoms. If the observations are made at the right time, the rather unusual character is very conspicuous since the tubes show reduction and acid production at the tops while at the bottoms there is no evidence of growth. With delayed observations the tubes are reduced, except for a pink band at the top, and coagulated throughout and show nothing unusual. The definite tendency of the organisms to begin their action at the tops of tubes of lit-

mus milk persists through many transfers and appears to be a well established character.

The cultures employed in the attempts to secure two types of lactic acid organisms from a common source were obtained from the unusual butter culture. They were run through several platings, using whey agar and an incubation of two days at room temperature, with the idea of establishing their purity.

PROCEDURE

The delayed growth of the organisms in the bottoms of the tubes, after rapid development at the surface, suggested that selective inoculations from the bottoms of the tubes before growth had developed too far might yield organisms having somewhat different characters than those secured from the surface.

The cultures from the bottoms were made by means of capillary pipettes having slowly tapering seals so that when the pipettes reached the bottoms the tips could be broken and material drawn into them. After removal from the material being cultured, the pipettes were sealed in a flame and then placed in a 1:1000 aqueous $HgCl_2$ solution to a depth greater than the depth of the milk in which they were used. After several minutes the $HgCl_2$ solution was wiped off with sterile cotton, the tips of the pipettes broken with a sterile forceps and the contents of each blown into a tube of sterile litmus milk. There was, of course, the possibility of carrying material from the surface down into the milk when a pipette was introduced, but the procedure was preferred to the use of special tubes made from test tubes by sealing a straight side arm into each near the bottom and culturing through this side arm. In all cases a culture was made from the surface before the bottom culture was taken so that there was an opportunity to compare the action of the organisms secured from the two portions of a tube.

All of the cultures were grown at a temperature of about 21°C. The freshly inoculated tubes were shaken vigorously to insure distribution of the inoculation material.

RESULTS OBTAINED

Most of the comparisons of material from the top and bottom of a culture were made on tubes that showed a pronounced change in the litmus milk at the surface but no change or only a slight reduction at the bottom. Some of the early attempts gave a conspicuous difference in the cultures from the two sources, those from the tops showing the first growth at the surfaces of the inoculated material and thus resembling the original cultures, while those from the bottoms showed the first growth in the lower portions of the tubes. However, this result was not always obtained and, in a considerable percentage of the trials, both the top and bottom material yielded cultures in which growth began at the surfaces of the inoculated milk. The observations suggested that there was more often a failure to get the two types of cultures when the tubes from which the inoculations were made showed definite reduction at the bottoms than when they did not. In some instances the carrying of surface material down into the milk when the pipettes were introduced may also have been a factor.

When the two types of cultures were secured by selective inoculations, they were frequently carried through series of transfers so that some idea of the stability of the characters could be obtained. The culture showing beginning development at the surface continued to give this change, as would be expected from the persistency of the character in the original cultures. The culture showing beginning development at the bottom usually gave the same change for a number of transfers but then rather gradually varied until it returned to the initial type and began growth at the surface. The time required for the reversion[1] varied considerably with different cultures. With some, only

[1] The change back to the initial type was regarded as a definite reversion, rather than the gradual dominance of the top form in a mixture, for the following reasons:

1. When the top type was known to be present in the material secured from the bottoms of tubes or any other source, it very quickly overgrew the other type and did not require several transfers for its dominance.

2. As pointed out later, the bottom type grew more slowly than the top type and should have been quickly submerged in mixtures of the two.

two or three transfers showed growth at the bottom first, while with others as many as eight transfers were made before the change back to the original type was noted. The effect of the interval between transfers on the time necessary for reversion was studied, using twenty-four- and forty-eight-hour periods. In general, about the same time was required although, of course, this involved twice as many transfers with the short period as with the long.

The cultures which showed the first reduction in the bottom of a tube of litmus milk grew somewhat more slowly than the cultures beginning their action at the top. This was found to be the case whether the inoculation material came from young or old cultures.

From time to time observations were made on the morphology of the two types of organisms. Both commonly showed chains; in young cultures these were often very long, while in older cultures they were shorter but there was still a very definite tendency to chain formation. In general, no difference in morphology between the two types of organisms could be noted, either in cultures showing only beginning reduction or in cultures after coagulation.

DISCUSSION OF RESULTS

From the results secured it appears that the *S. lactis* cultures studied, which were unusual in that they began growth in tubes of litmus milk at the tops instead of the bottoms, could be split into two types, one of which differed definitely from the original cultures by beginning its growth at the bottoms of tubes of litmus milk. Although the variation secured by selective inoculation resulted in a culture that was more like the usual *S. lactis* culture than was the original, it apparently was unstable and, after a number of transfers, reverted to the original type. This suggests that each of the types of organisms encountered can split off from the other. The eventual dominance of the type developing first at the surface of milk is undoubtedly, in part, due to its comparatively rapid growth so that the other type tends to remain submerged to such an extent that it is not com-

monly evident without selective inoculations.   Data reported by Hammer[2] show that certain ropy *S. lactis* cultures were split into ropy and non-ropy types by plating and picking colonies.   In one instance ropy cultures were secured from a non-ropy one which indicates that this variation was also a reversible one.

The variations that appear to be rather common with *S. lactis* organisms may be a factor in explaining the differences that are encountered among cultures belonging to this species.   Probably most of the variations that occur never become conspicuous because of some definite factor, such as competition from the original type, but it would be expected that occasionally the variant would outgrow the other type and thus become of real significance.

[2] Hammer, B. W.   Studies on ropiness in cultures of *Streptococcus lactis*. Iowa Agr. Expt. Sta. Res. Bul. 74, 1923.

OBSERVATIONS ON ROPINESS IN BUTTER CULTURES*

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Ropiness in milk resulting from the activity of *Streptococcus lactis* is of special importance in the dairy industry because of its occurrence in butter cultures under both practical and carefully controlled laboratory conditions. Cultures which have been normal in consistency for extended periods may develop a ropy condition, either gradually or suddenly, and then on plating commonly yield some *S. lactis* cultures that produce ropiness in milk (*S. lactis var. hollandicus*) (3). The ropy condition in the butter culture may disappear and then reappear later; with certain cultures the appearance and disappearance of the ropiness, at various intervals, continues for long periods without apparent related causes.

From the data available it appears that ropiness in *S. lactis* cultures is not accompanied by a fixed group of other characters (3). This leads to the suggestion that the ropiness may be the result of different types of variations in cultures or, stated conversely, that variations of different types may result in a culture developing ropiness. The data herein reported were secured during the investigation of a ropy butter culture with which the air requirements of the organism seemed to be related to the ropiness.

HISTORY OF THE BUTTER CULTURE STUDIED

The butter culture studied was originally secured by combining *S. lactis* and *Streptococcus citrovorus*. It was carried for several years with very satisfactory results and was sent to many creameries in various parts of the United States. The usefulness of the culture in producing a desirable flavor and aroma in butter is shown by the fact that it was employed in many churnings winning prizes in state and national butter scoring contests.

After its value was recognized, subcultures were made from time to time and each of these was carried through its own series of transfers with the object of being reasonably certain that the butter culture would not be lost.

In the late summer of 1928, the subcultures that were being carried in the laboratory under very careful conditions suddenly began to show slight ropiness. In some instances transfers sent to creameries developed ropiness, while in others they did not. Later the laboratory transfers showed a pronounced ropiness. Continued transferring finally resulted in the disappearance of the condition and during the nine months that have elapsed since this disappearance there has been no tendency for it to reappear.

Before ropiness developed in the butter culture at the Iowa Agricultural Experiment Station, a transfer was sent to Dr. H. Macy at the Minnesota Agricultural Experiment Station. Doctor Macy reported that, after a considerable number of satisfactory transfers, ropiness appeared in the culture carried by him. This was at about the same time that the ropiness appeared at the Iowa station.

<div align="center">RESULTS OBTAINED</div>

<div align="center">*S. lactis organisms present in the ropy butter culture*</div>

A transfer of the butter culture which had developed the ropy condition was plated on whey agar and, after incubating the plates at room temperature for two days, 14 *S. lactis* colonies were picked into litmus milk; one of these produced a very ropy condition, while the others showed no ropiness. Another transfer that was plated yielded 2 cultures producing ropiness in milk out of the 33 *S. lactis* cultures picked. Additional attempts to isolate ropy *S. lactis* cultures by plating transfers of the ropy butter culture also indicated that they were much less numerous than the non-ropy cultures and, in some instances, the ropy cultures were entirely missed.

The ropy or non-ropy character of the *S. lactis* cultures isolated appeared to be very definite since, in a number of trials, all the colonies picked into litmus milk from plates poured with one of

TABLE 1

General difference in the development in litmus milk of ropy and non-ropy S. lactis cultures from one source

Inoculated at 8 p.m.   Incubation at room temperature

CULTURE	TIME OF INCUBATION						
	13 hours	14 hours	15 hours	16 hours	17 hours	18 hours	19 hours
Ropy 1	Slight reduction at surface	Top ½ reduced	Top ¾ reduced	Top ⅝ reduced	Top ⅒ reduced	Reduced except pink at surface	
Ropy 2	No change	Slight reduction at surface	Slight reduction at surface	Slight reduction at surface	Slight reduction at surface	Reduced except at bottom	Top ½ reduced, pink at surface
Ropy 3	Slight reduction at surface	Slight reduction at surface	Top ¼ reduced	Top ¼ reduced	Top ½ reduced	Top ¼ reduced	Top ½ reduced
Ropy 4	Slight reduction at surface	Slight reduction at surface	Slight reduction at surface	Reduced at surface	Reduced at surface	Top ⅔ reduced	Top ¾ reduced
Non-ropy 1	No change	No change	No change	Reduced at bottom	Reduced completely	Reduced except pink at surface	
Non-ropy 2	Slight reduction at bottom	Reduced except at surface	Reduced except at surface	Reduced completely	Reduced completely	Reduced except pink at surface	
Non-ropy 3	No change	No change	Bottom ⅔ reduced	Bottom ⅔ reduced	Reduced except at surface	Reduced except pink at surface	
Non-ropy 4	No change	No change	No change	Slight reduction at bottom	Slight reduction at bottom	Reduced except pink at surface	

the cultures gave the same change in milk as the culture plated. These results indicated that there was no regular transition from either a ropy or non-ropy *S. lactis* type to the other.

Comparisons of the morphology of the ropy and non-ropy *S. lactis* cultures in milk suggested that there was somewhat more of a tendency to chain formation with the ropy than with the non-ropy cultures. The difference was not clear-cut, however, and some chains were present in the non-ropy cultures, while pairs were common in the ropy ones.

There was a conspicuous difference between the ropy and non-ropy cultures in the portion of the tubes of litmus milk in which growth began. While the non-ropy cultures showed the first reduction at or near the bottom of a tube, as is most often the case with *S. lactis*, the ropy cultures began their reduction at the surface. Occasionally with the latter the growth was so definitely limited to the surface for a time that the milk was curdled there when it was not even reduced in the bottom of the tube. The milk inoculated with the ropy cultures eventually curdled throughout just as did that inoculated with the non-ropy cultures.

The general difference in the development of the two types of cultures in tubes of litmus milk is shown in table 1. The cultures were inoculated at 8 p.m., the inoculated milk thoroughly shaken and observations made the next day; incubation was at room temperature. From the results given, it is evident that the two types of cultures differed very definitely in the portions of the tubes in which growth occurred first. The non-ropy cultures began the reduction at the bottom and extended it from that area while the ropy cultures began their growth at the surface and then developed downward. Additional observations, also made under comparable conditions, confirmed the general variation shown in table 1.

Selective transfers of material from the butter cultures

The tendency of the ropy *S. lactis* cultures to develop at the surface suggested attempts to isolate them from the butter culture by a series of transfers, using surface material for the inoculations. Because the butter culture had lost its ropy

character, it was necessary to try this procedure on a non-ropy transfer. It was inoculated into a tube of litmus milk and, after coagulation, a series of transfers through tubes of litmus milk was begun, the inoculation material being taken regularly with a needle from the very surface of the fermented milk. The transfers were made every day or two, depending on the rate of coagulation, and were incubated at room temperature. After somewhat more than a month of such transferring, slight ropiness was noted, soon there was evidence of reduction beginning at the surface and then pronounced ropiness developed. On continued transferring the ropiness and early surface growth were maintained through many inoculations.

In another more extended trial, that was also begun with the butter culture showing no evidence of ropiness, room temperature and 21°C. were used and at each temperature one series of transfers involved inoculations with a needle from the surface of the fermented milk, while in another series the transfers were made by dipping a loop well down into the milk. At room temperature the eighth surface transfer was definitely ropy and at about the same time reduction regularly began at the top. This combination of characters persisted through the remainder of the 88 transfers made, although some of the later inoculations involved the transferring of material several days old. None of the 88 deep transfers made at room temperature became ropy or began the reduction of the milk at the surface. Microscopic examinations showed that after a number of transfers there was much more of a tendency to chain formation with the organisms in the material secured by surface inoculations than in that secured with deep transfers. Volatile acid determinations were made on material from the two series after 75 inoculations of each; the method employed was the one regularly used at the Iowa Agricultural Experiment Station (2). The value for the surface material was 2.9[1] and that for the deep material 20.7; these

[1] This value represents the cubic centimeters of N/10 NaOH required for the neutralization of the first 1000 cc.of distillate obtained when a 250-gram portion of the fermented milk was distilled with steam after the addition of 15 cc. of approximately N/1 H_2SO_4.

developing in the side arm when the incubation period was prolonged.

Some of the non-ropy cultures secured were carried through a number of transfers without ropiness appearing, while others soon developed a ropy condition. When ropiness appeared, it was usually slight in one transfer and then more pronounced in a later one.

DISCUSSION OF RESULTS

The isolation of ropy *S. lactis* cultures (*S. lactis var. hollandicus*) from a ropy butter culture is in agreement with the evidence from various sources indicating that ropiness in butter cultures is most often due to a ropy *S. lactis* organism instead of to one of the types commonly related to ropiness in sweet milk. The ropy organism isolated, however, differed from most *S. lactis* strains in the tendency to begin the reduction of tubes of litmus milk at the surface instead of at the bottom.

The development of ropy cultures by selective inoculations from the surface of a butter culture that had lost its ropiness, after a period when this character was regularly evident, was undoubtedly due to the correlation between the ropy condition and the air requirements. The ropy cultures secured were apparently cultures of *S. lactis* only, since volatile acid determinations showed that the volatile acid producers associated with *S. lactis* in butter cultures were not present. Presumably the ropy *S. lactis* organism, after a period when it was sufficiently numerous to cause ropiness in the butter culture, decreased to a point where the ropiness disappeared. For a time after this decrease, the organism could still be isolated by selective inoculation, which was easily carried out because of the unusual air relationship, but later it had either entirely disappeared or decreased to such a point that the selective procedure failed.

The development of non-ropy cultures from ropy ones by using material showing beginning reduction in the bottoms of the tubes of litmus milk again shows the correlation between the ropiness and the air requirements. It further illustrates the splitting off of a non-ropy culture from one that is definitely ropy and, in this

respect, confirms the work of Hammer (1) who found it possible to secure both non-ropy and ropy *S. lactis* organisms by picking colonies from plates poured with certain ropy *S. lactis* cultures.

Two explanations for the original presence of *S. lactis var. hollandicus* in the butter culture suggest themselves; first, contamination and, second, a change in the character of an organism already there.   Contamination does not seem probable because of the other butter cultures that remained normal when handled under the same conditions and also because the most logical sources of contamination with butter cultures would not be expected to supply *S. lactis* of any type.   In the work reported by Hammer (1), ropy *S. lactis* cultures were secured in one instance by plating a non-ropy culture and picking colonies into litmus milk, and such a sudden change in the character of an organism seems the most logical explanation in the case of the butter culture studied.   There would not need to be a change in a large number of cells because a variation in a few or even one cell could become of importance through reproduction.

Presumably a change in the character of an organism has a definite cause.   In the instance studied, the factor responsible for the ropiness seems to have definitely influenced the air requirements and since in at least some instances the ropy cultures showed more chain formation than the non-ropy ones, it may have affected the morphology.

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MINUTES OF MEETING OF ADVANCED REGISTRY SECTION, AMERICAN DAIRY SCIENCE ASSOCIATION

HOTEL MISSOURI, ST. LOUIS, MISSOURI

8:15 P.M. October 14, 1929

Chairman, J. R. DICE   *Acting Secretary,* K. S. MORROW

Minutes of last meeting read and approved. The chairman appointed the following nominating committee: W. E. Peterson, Minnesota; R. T. Harris, Wisconsin.

Professor J. B. Fitch, chairman of the Breeds' Relations Committee, gave the following report:

REPORT OF THE BREEDS' RELATIONS COMMITTEE MEETING HELD OCTOBER 13, 1929; ST. LOUIS, MISSOURI

The Summary of answers to letters in regard to Advanced Registry rules was read and discussed. The comment of breed association officials to this letter was not enclosed in the letter sent to the supervisors of Advanced Registry. Some of the comments were as follows:

1. That the no talking rule was not being enforced in many of the states.

2. That there was a wide difference in the method of handling finances at the different schools and a lack of business methods in handling accounts at some institutions. It was brought out that the Jersey and Guernsey breeds guarantee payments up to three months, while the Ayrshire and Holstein breeds extend this to six months.

3. That the preliminary milking was not recorded or tested in many states.

If a state has adopted the uniform rules of the American Dairy Science Association there should be no question about the enforcement of rules covering comments 1 and 3 above.

A committee is to be appointed to make recommendations

regarding the guarantee for payment of testing. This matter is becoming more complicated due to the adoption of the bimonthly test.

The Breeds' Relations Committee approved the use of the bimonthly test and suggested that the breed associations consult with the representatives of the American Dairy Science Association in formulating their rules for this test.

R. T. Harris of Wisconsin reported for the committee on the uniform blank for herd tests. A uniform blank has been agreed upon and will be printed, along with the two-day blanks. As the supply of two-day report blanks is low, plans were made to have a new supply of this blank and the uniform herd test blank printed in the near future.

Motion made by J. E. Wylie, Tennessee, and seconded by R. B. Becker, Florida, that report of Breeds' Relations Committee be approved and adopted as reported. Motion carried.

Warren Gifford, Missouri, presented a paper entitled, "A Statistical Study of the Reliability of Bimonthly Tests."

Lynn Copeland, American Jersey Cattle Club, presented a paper entitled, "The Bimonthly Test," Mr. Copeland reported that 77 Jersey breeders were at the present time using this test.

The meeting was opened for discussion of the papers presented by Gifford and Copeland. Eight states reported the use of the Bimonthly test.

H. W. Norton, Jr., Holstein-Friesian Association of America, explained briefly the purpose and operation of the Holstein Plan of Herd Classification. He reported that 63 herds, totaling 1800 animals, had been classified to date.

Wm. Moscrip, Holstein-Friesian Association of America, led the discussion following Norton's remarks. He especially mentioned the aim of the Holstein-Friesian Association to eliminate from the Holstein breed those animals falling in the two lower classifications of the plan.

The Nominating Committee reported the following names:
Chairman: A. A. Borland, Pennsylvania; E. L. Anthony, Michigan.

Secretary: P. C. McGilliard, Oklahoma; A. F. Kuhlman, Illinois.

J. E. Wylie, Tennessee, moved and C. L. Allen, New York, seconded the adoption of the report of Nomination Committee. Motion carried.

The following were elected: Chairman, E. L. Anthony; Secretary, P. C. McGilliard.

THE RELIABILITY OF BI-MONTHLY TESTS*

WARREN GIFFORD†

University of Missouri

The problem of obtaining an accurate quantitative measure of the dairy cow's inherited producing ability has occupied the attention of dairymen since very early times. According to Morse (1), perhaps Pyrrhus, in 300 B.C., who measured milk from cows that produced approximately 40 liters per day was one of the first to realize the importance of such a measure. The great developments in tests and measures that have adapted themselves to such a purpose have been made during the past half century.

The strictly official plan of testing, as accepted at the present time by the Holstein-Friesian Association of America (2), has met the requirements as a test that is accurate for the period of time that it is put into operation and has eliminated to a great extent, fraudulent practices that may occur in the making of records.

Although the strictly official test plan has met the requirements of an accurate quantitative measure of the dairy cow's producing ability under certain conditions of management and feeding, it has not met with favor among dairy cattle breeders. It has been shown that short time official records are of relatively little value in predicting a cow's milk and butterfat production for a complete lactation period, and the enormous costs incurred in the supervision of the long-time strictly official test have made it an impractical measure of milk and butterfat production.

In order to reduce the cost of testing, breed associations have adopted plans of supervision that determine the amounts of butterfat produced during a short interval each month, i.e.,

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one and two days, and at which time considerable effort is expended in determining whether or not the records are honestly made and accurately reported, and from these data the amounts of fat produced during the lactation period have been estimated. The reliability of these tests has been studied by Yapp (3), Petersen (4), and others, and it was shown that the errors of estimate were rather small but that considerable variations frequently occurred.

These forms of records, known as semi-official records, have been great factors in the improvement of pure bred dairy cattle. Since there are twelve or more supervisions during the lactation period, there has been considerable expense to the breeders who have conducted these tests, and no doubt many breeders of purebred dairy cattle have not been able to meet the financial obligations entailed in testing. Therefore, some of the advancement that could have been made in the improvement of dairy cattle has been curtailed.

It has been suggested from time to time that any method of supervision of yearly tests that would reduce the cost of testing, give an accurate estimate of the yearly production, and still not lower the standards of the present plans of testing by giving greater opportunity for fraudulent practices, would act as a stimulus to the amount of testing.

McDowell (5) and Copeland (6) have presented data to show the relative reliability of yearly records that have been estimated from six supervisions per year. Gaines (7) has shown that when the short-time official test is conducted during the fifth month of the lactation period, it is of greater reliability than other short time official tests. Saiz (8) has also presented a plan of three tests per year that will estimate the annual production with considerable accuracy.

Copeland selected at random 250 Register of Merit records made under monthly two-day supervision and 250 records that were made by the one-day supervision; and computed the yearly butterfat records by using only the alternate regular tests and the automatic retests which those tests occasionally required. In 50 per cent of the cases the first test made after the starting

of the lactation period was used as a beginning and in the other 50 per cent the second test was used.   He found that the average variations were as follows:

250 records (two-day supervision)............ ... .$\begin{cases} 6 & 73 \text{ pounds fat} \\ 0 & 901 \text{ per cent} \end{cases}$

250 records (one-day supervision)....................$\begin{cases} 7 & 69 \text{ pounds fat} \\ 1 & 53 \text{ per cent} \end{cases}$

500 records (one- and two-day supervision...........$\begin{cases} 7 & 21 \text{ pounds fat} \\ 1 & 21 \text{ per cent} \end{cases}$

From the study of these data he concluded that records that were made every other month under supervision show little variation from those made by the usual monthly supervision.   McDowell drew similar conclusions from his study of Cow Testing Association records.

In view of the findings of the studies of the Register of Merit records, the American Jersey Cattle Club adopted the plan of Bi-monthly testing.   Other breed associations have been considering the adoption of a similar plan of Advanced Registry.

Therefore, these interests have inspired the writer to make some investigations as to the reliability of butterfat records obtained by the bi-monthly plan of testing as compared to the present form of monthly testing for yearly records.

The records studied were obtained by selecting at random 100 or more Advanced Registry records for Holstein and Guernsey cows tested monthly by the two-day test plan for each of the following groups of yearly butterfat production:

300 to 399.9	700 to 799.9
400 to 499.9	800 to 899.9
500 to 599.9	800 to 999 9
600 to 699 9	1000 and over

It was believed that the selection of records representing definite classes of production was of special value in getting data that would be representative of all breeds and such that would show the reliability of the method of testing for records of cows at various productions.

From these data yearly records were estimated by applying the bi-monthly test plan, using only alternate tests. The records obtained by using the tests for the first, third, fifth, seventh, ninth, and eleventh months were called the "odd month records" and those that were calculated by starting the test with the second month of the lactation period and continuing by using the

FIG. 1. DISTRIBUTION OF THE DEVIATIONS IN POUNDS OF FAT BETWEEN MONTHLY AND ESTIMATED BI-MONTHLY RECORDS IN THE 300- AND 400-POUND GROUP

fourth, sixth, eighth, tenth, and twelfth month tests, respectively, were called the "even month records."

For convenience, these records were combined into four groups as follows: those between 300 and 499 pounds, 500 and 699 pounds, 700 and 899 pounds and those above 900 pounds.

In order to compare the reliability of the estimated bi-monthly yearly records as calculated, the deviations in pounds of fat from the records as determined by the monthly tests were determined,

and the frequencies plotted, and are shown in figures 1 to 5, inclusive.

The standard errors of estimate were determined and the correlation coefficients between the actual records and the estimated records were computed for all the groups and for the group representing all records studied, and are given in table 1.

FIG. 2. DISTRIBUTION OF THE DEVIATIONS IN POUNDS OF FAT BETWEEN MONTHLY AND ESTIMATED BI-MONTHLY RECORDS IN THE 500- AND 600-POUND GROUP

The value of the means for these groups indicate whether or not the estimations are satisfactory in determining the yearly butterfat records. The standard error of estimate is a measure of the prediction value of the method used for estimating the production and indicates the variation that may be expected in the application of the method. About 69 per cent of all of the bi-monthly records will lie within the range of the standard error of estimate, 95 per cent will fall within two times the standard
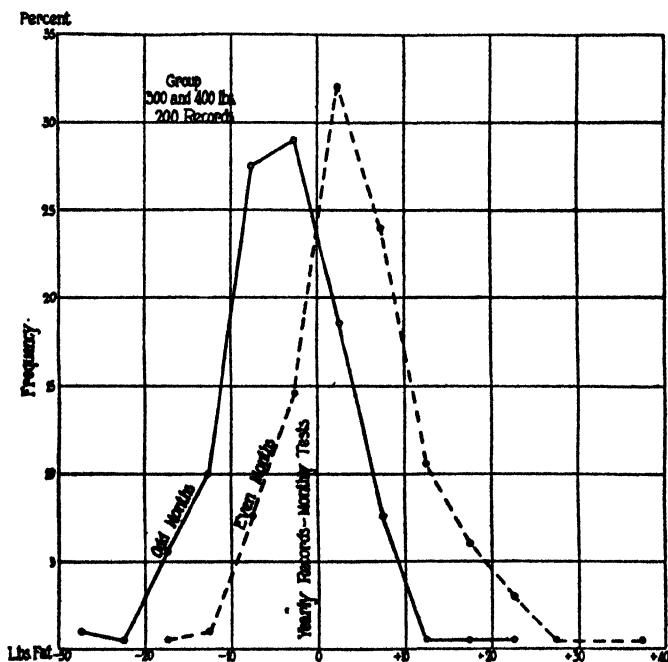
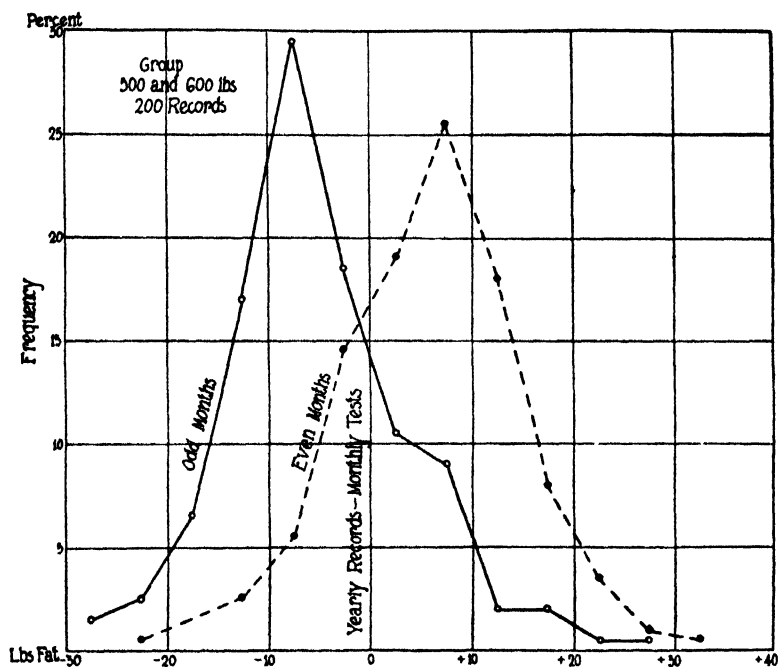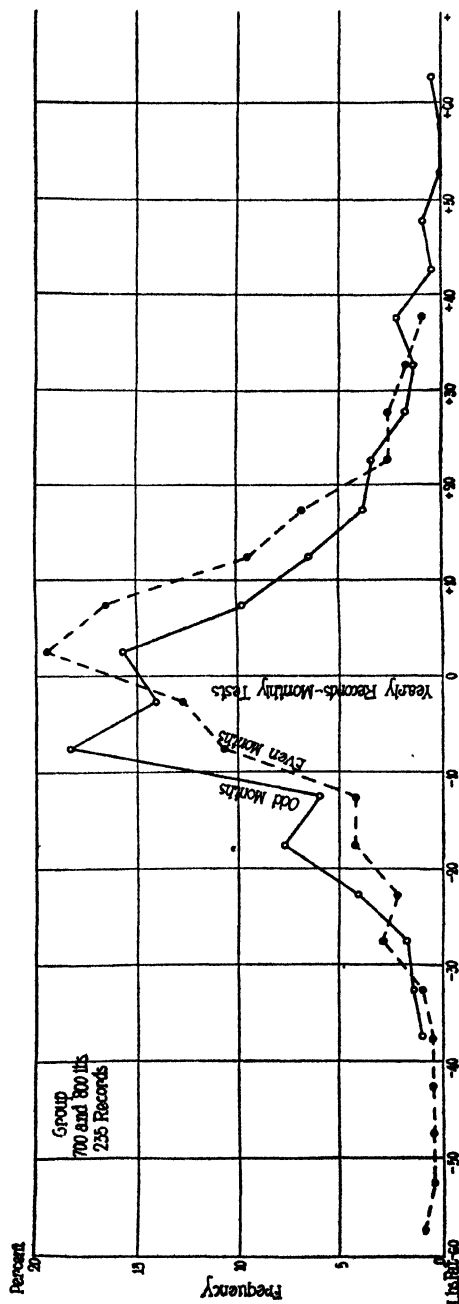FIG. 3. DISTRIBUTION OF THE DEVIATIONS IN POUNDS OF FAT BETWEEN MONTHLY AND ESTIMATED BI-MONTHLY RECORDS IN THE 700- AND 800-POUND GROUP
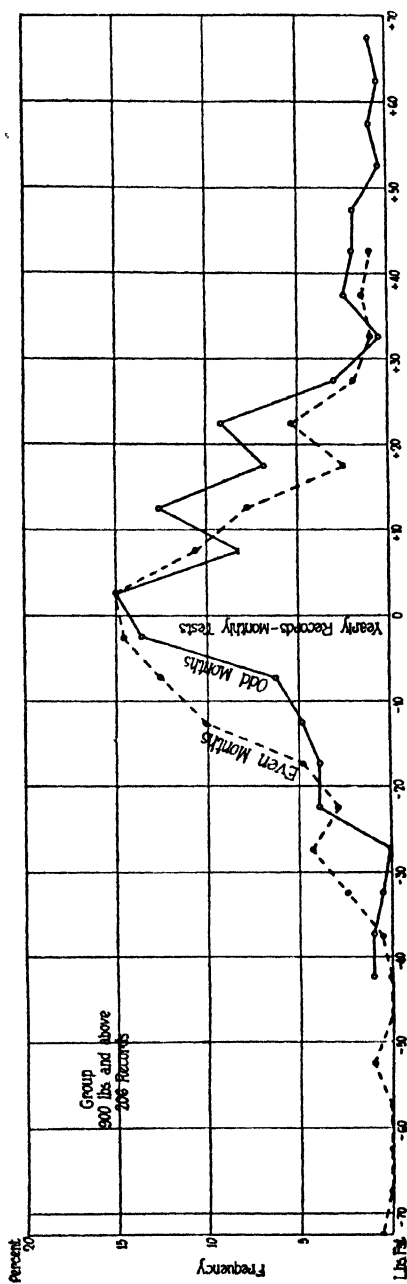
FIG. 4. DISTRIBUTION OF THE DEVIATIONS IN POUNDS OF FAT BETWEEN MONTHLY AND ESTIMATED BI-MONTHLY RECORDS IN THE 900 POUNDS AND ABOVE GROUP
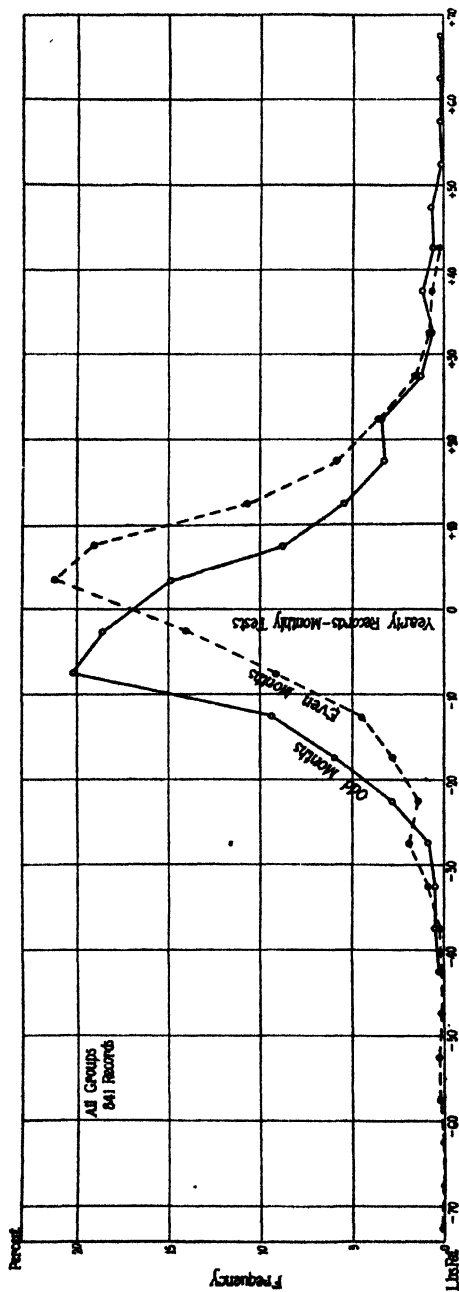
Fig. 5. Distribution of the Deviations in Pounds of Fat between Monthly and Estimated Bi-monthly Records in all Groups of Records

error of estimate, and 99.7 per cent will fall within three times the standard error of estimate. Therefore, the smaller the standard error of estimate the less the dispersion about the line of the actual records.

An examination of the data presented in table 1 shows that the means of the deviations from the actual records for the odd month for the 300 and 400 pound groups are -4.12 ± 0.371 and

TABLE 1

Means, variables, and coefficient of correlation between yearly butterfat records estimated by the bi-monthly test plan from the records obtained by monthly tests

FACTORS	NUM-BER YEARLY REC-ORDS	CLASS		MEAN OF THE DEVIATION	STANDARD ERROR	COEFFICIENT OF CORRELATION r
		pounds		*per cent*		
	200	300, 400	Odd	-4.12 ± 0.347	7.27 ± 0.245	*0.981 ± 0.002
			Even	$+4.82 \pm 0.371$	7.78 ± 0.262	0.991 ± 0.001
	200	500, 600	Odd	-5.12 ± 0.435	9.12 ± 0.308	0.982 ± 0.002
			Even	$+5.80 \pm 0.427$	8.95 ± 0.302	0.986 ± 0.007
Monthly bi-monthly records	235	700, 800	Odd	-0.1 ± 0.694	15.78 ± 0.491	0.972 ± 0.003
			Even	$+0.97 \pm 0.667$	15.16 ± 0.472	0.973 ± 0.002
	206	900 and above	Odd	$+7.33 \pm 0.894$	19.02 ± 0.632	0.956 ± 0.004
			Even	-1.28 ± 0.767	16.32 ± 0.542	0.971 ± 0.003
	841	All groups	Odd	-4.90 ± 0.346	14.88 ± 0.244	†0.986 ± 0.001
			Even	$+2.50 \pm 0.375$	13.23 ± 0.218	0.997 ± 0.001

Class interval:
* 10.0 pounds.
† 20.0 pounds.

-5.80 ± 0.427 pounds fat, and $+4.82 \pm 0.371$ and $+5.80 \pm 0.427$ pounds fat for the even month groups, respectively, and a total difference of 8.9 and 10.9 pounds of fat between the means of estimated records calculated on odd and even months. The standard errors of estimate are relatively small, being 7.275 ± 0.245 and 9.123 ± 0.308 pounds for the odd month records and 7.782 ± 0.262 and 8.950 ± 0.302 pounds for the even month records.

The means for the 500 and 600 pound groups indicate that the method used in predicting the yearly records was even more satisfactory since the means of the deviations for the odd month records are -0.1 ± 0.694 and 7.33 ± 0.894 pounds and for the even months $+0.97 \pm 0.667$ and -1.28 ± 0.767 pounds, respectively. The standard errors for these groups were 15.980 ± 0.491 and 19.015 ± 0.632 pounds for the odd month records and 15.165 ± 0.472 and 16.315 ± 0.542 pounds for the even month records.

When all of the data are combined, there is a mean of -4.9 ± 0.346 pounds fat for the deviations of the records estimated by the odd month test and $+2.499 \pm 0.375$ pounds fat for those estimated by using the even month tests.

The coefficients of correlation indicate that there is a significant degree of relationship between all groups of estimated records and the yearly records obtained by the monthly plan of testing.

Therefore, it seems, according to the above data, that the bimonthly method of estimating the yearly fat production of dairy cows is satisfactory, when such records are made with the same degree of accuracy and honesty as are those records made by the monthly test plan.

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THE EFFECT OF LACTOSE ON THE SURVIVAL OF ESCHERICHIA COLI WHEN HEATED TO 145°F. FOR THIRTY MINUTES*

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In commercial pasteurization the evidence is conclusive that members of the Escherichia-Aerobacter group may survive in milk that has been heated to approximately 145°F. for a period of thirty minutes. This temperature has been officially accepted as being sufficiently high to insure the safety of pasteurized milk.

It is rather uncertain whether the survival of members of this group is due to defective pasteurizing equipment, to the physiology of the organism, or to certain biochemical influences of the milk itself. Considering the colloidal state and chemical constituents of milk, it would seem possible that a protective action may at times be established.

Of the chemical constituents in milk lactose offers the best possibility for investigation. This sugar is present in cow's milk in amounts varying between 4 and 5 per cent.

The available literature on this subject supports the evidence herewith presented. Robertson[1] states that,

Hypertonic solutions, as indicated by comparisons of heating cells in nutrient broth containing increasing concentrations of sucrose, have a protective action up to and including 50 per cent sucrose. The protective action may be explained, in part, by the osmotic influence of the sucrose solution outside of the cell wall, and, in part, by the presence of capsules formed about the cells when growh in the sucrose solutions.

EXPERIMENTAL

Forty-eight hour broth cultures of the strains to be tested were made from agar slants. One-tenth cubic centimeter quantities

were inoculated into tubes of broth containing 0, 1, 2, 3, 4, 5, 7.5, 9.5, 12.5, 15, and 20 per cent lactose and then incubated at 37°C. for forty-eight hours.   One-tenth cubic centimeter quantities were then inoculated into corresponding tubes of lactose broth and pasteurized at 145°F. for thirty minutes in a water bath.   The tubes were removed, cooled, and the contents of each poured into fermentation tubes containing 2 per cent lactose

TABLE 1

Influence of lactose on the survival of Escherichia coli heated to 145°F. for thirty minutes

+ growth; − no growth

	TEST NUMBER	0	1 PER CENT	2 PER CENT	3 PER CENT	4 PER CENT	5 PER CENT	7.5 PER CENT	9.5 PER CENT	12.5 PER CENT	15 PER CENT	20 PER CENT
	1	−	−	−	−	+	+	+	+	+	+	+
	2	−	−	−	−	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+
	4	−	−	−	+	+	+	+	+	+	+	+
Strain 1...........	5	−	−	+	+	+	+	+	+	+	+	+
	6	−	−	−	+	+	+	+	+	+	+	+
	7	−	−	−	−	+	+	+	+	+	+	+
	8	−	−	−	+	+	+	+	+	+	+	+
	9	−	−	−	−	+	+	+	+	+	+	+
	1	−	⇀	+	+	+	+	+	+	+	+	+
Strain 2...........	2	−	−	−	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+
	1	−	−	−	−	+	+	+	+	+	+	+
Strain 3...........	2	−	−	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+

broth.   In addition, 1 cc. portions from each tube were plated on lactose agar.   Results were recorded after ninety-six hours incubation at 37°C.

DISCUSSION

Table 1 presents data showing the protective action of increasing concentrations of lactose on the survival of *Escherichia coli*

when heated to 145°F. for thirty minutes. A protective action is established when these organisms are grown in broth containing from 4 to 20 per cent lactose. In several cases survival occurred in as low as 2 or 3 per cent solutions of lactose. As the percentage of lactose in milk varies between 4 and 5 per cent a similar phenomenon may occur in pasteurized milk.

The thermal death-points of the strains used had been determined to be between 142° and 143°F. for thirty minutes. The tubes of broth containing no lactose acted as controls. Comparing these temperatures with the one at which survival occurred, that is, 145°F. for thirty minutes, it is seen that the increasing concentrations of lactose may cause an increase in the death-point temperatures of at least 2° or 3°. This factor may prove to be vital in commercial pasteurization.

The data in table 1 are based on 15 determinations. Three trials not included showed this protective action but with breaks.

The findings indicate that the greater the concentration up to and including 20 per cent lactose, the greater the probability for survival. This fact may be of value in explaining the survival of members of the Escherichia-Aerobacter group in pasteurized ice cream mixes which usually contain from 10 to 15 per cent sugar. It should be noted that the sugar used in ice cream mixes is sucrose but the same protective action may occur due to the fact that lactose and sucrose are isomeric.

<div align="center">CONCLUSION</div>

1. The ability of *Escherichia coli* to survive pasteurization when grown in increasing concentrations of lactose suggests the possibility of a similar protective action in milk.

2. This survival of *Escherichia coli* seems to bear a relation to the concentration of lactose up to and including 20 per cent.

3. Survival of members of the Escherichia-Aerobacter group in pasteurized ice cream mixes may be caused by the protective action of the high sugar content.

THE ESCHERICHIA-AEROBACTER GROUP AS AN INDEX TO PROPER PASTEURIZATION*

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Several investigators have attempted to use the survival of certain organisms in milk as an index to the efficiency of pasteurization. The coli bacillus, because its thermal death-point more closely approaches the pasteurization temperature, has been often used in this capacity. Other investigators have been reluctant in accepting the data offered to support the coli index of pasteurized milk. The problem is still in a state of controversy because of the need of additional work.

Several influencing factors are brought into prominence when heat, especially high temperatures, is applied to protoplasmic matter. Cell metabolism is materially increased resulting in the possible formation of a resistive cell wall, an alteration of the protoplasm, or a rearrangement of the molecular structure of the cell. Acclimatization of bacteria to high temperatures may occur if successive generations are grown from those having high absolute thermal death-points or those possessing true heat-resistance such as the thermophiles. It is logical that the effects of these factors must be thoroughly investigated and considered before any relationship can be established between the coli count and the efficiency of pasteurization.

REVIEW OF LITERATURE

Coli bacilli with high thermal resistances have been observed by De Jong and De Graff (7) who described seven strains of *E. coli* which survived 149° to 152.6ʻF. for thirty minutes in milk or broth. One strain withstood 185°F., frequently it survived 140° to 149°F. for thirty minutes. The culture was more easily killed in bouillon than in milk. They concluded from these

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results that the presence of *E. coli* in pasteurized milk could not be taken as an index of proper pasteurization. Gage and Stoughton (3) showed that the majority of thermal death-points of *E. coli* were from 132° to 140°F. The temperature at absolute death-point showed the presence of heat resistance organisms in this group. Zelenski (12) also found a strain of *E. coli* which survived unusually high temperatures.

Ayres and Johnson (2) observed that the thermal death-points of 174 cultures of the coli bacillus isolated from cow feces, milk, cream, human feces, flies, and cheese, varied considerably when heated in milk for thirty minutes. At 140°F., 95 cultures (54.69 per cent) survived. One culture was not killed at 150°F. on one occasion only. All cultures had low majority death-points, survival was due to the resistance of a few cells. This fact complicated the coli test as an index to the efficiency of pasteurization. They state that the coli test may be of value if the milk is pasteurized at 150°F. for thirty minutes.

Ayres and Johnson (1) pointed out the relationship between the "majority" and "absolute" death-points of bacteria in relation to pasteurization. These investigators found the "majority" death-point of *A. aerogenes* to be 135°F. for thirty minutes, while the "absolute" point was a little above 150°F. for thirty minutes. When 135°F. was used surviving cells could be detected by examining 1 per cent of the volume pasteurized, when 140°F. was used 2 per cent of the volume was tested in order to find those surviving: at 145°F. no living organism could be detected in 1 cc. or even when 14 per cent was tested, surviving cells were found in the remaining 85 per cent.

Tanner (11) states that,

Although neither the *Escherichia coli* (*Bacterium coli*) cultures nor the *Serratia marcescens* (*Bacillus prodigiosus*) strains used proved to be very good as indicators of efficiency of pasteurization by the holder process, for several reasons the *Escherichia coli* (*Bacterium coli*) would probably prove to be more accurate as such an indicator. It seems fair to assume, in view of the results obtained, that *Escherichia coli*, in concentrations in which it occurs in milk, would be destroyed by exposure to 62.8°C. for thirty minutes, yet there is always the possibility

of encountering resistant strains or cultures containing some resistant cells.

SCOPE OF PROBLEM

The scope of this paper includes the determination of the presence or absence of organisms of the Escherichia-Aerobacter group in raw milk and in milk pasteurized under commercial conditions. Data were collected to determine whether or not coli bacilli survived the pasteurization temperatures.

Part of the studies were performed with pure cultures under laboratory conditions so actual associative and environmental conditions were disregarded. The conclusions may be, as stated by Marshall, "Like studying man apart from society in order to obtain his social relations." However, until better methods of technique are perfected to obtain results under actual conditions, we must rely on laboratory tests and pure cultures to assist us in obtaining the information that we are seeking.

All samples of milk were obtained from the dairy of the University of Maryland. The experiments were conducted from August, 1927, to April, 1929 under the supervision of Dr. E. M. Pickens, Head of the Department of Bacteriology.

METHODS AND PROCEDURE

The samples of milk to show the efficiency of pasteurization were taken from the vat before and immediately after the heating process. Pasteurization was accomplished by heating in a Pfaudler glass-lined pasteurizer to approximately 145°F., holding for thirty minutes and then cooling to about 50°F.

These samples of milk were plated on standard agar in the following manner: the raw milk in dilutions of 1:100; 1:1000; 1:10,000; the freshly pasteurized milk in dilutions of 1:10; 1:100; and 1:1000. An Endo's plate was made from each sample in a dilution of 1:10. Ten cubic centimeters, 1 cc.; and 0.1 cc. portions of milk from each sample were inoculated into fermentation tubes containing 2 per cent lactose. All plates and tubes were incubated at 37°C. for forty-eight hours.

The agar used was the standard beef extract media adjusted

to a pH of 6.5 to 6.6 as recommended by Standard Methods of Milk Analysis (10). The Endo's media was prepared from the formula recommended in Giltner's Microbiology (4).

The technique used to determine the possible acclimatization of coli organisms to high temperatures consisted of two parts; the determination of the thermal death-points of the strains to be used, the actual acclimatizing process. In the first part, characteristic coli colonies were isolated from raw milk plates and replated several times to insure the purity of the cultures. These strains were then inoculated into dextrose, sucrose, lactose, and mannite broths to test their gas-forming abilities.

To differentiate these strains as fecal or non-fecal types, the Voges-Proskauer and methyl red reactions were used. The first test was performed by growing the strains in 5 cc. quantities of Bacto M. R.-V. P. medium for twenty-four hours after which equal amounts of 10 per cent KOH were added. Fecal types are Voges-Proskauer negative. The methyl red tests were performed by growing the strains in 5 cc. quantities of Bacto M. R.-V. P. medium for forty-eight hours after which 5 drops of methyl red were added.

To determine the thermal death-points of the selected strains the open tube method was used. A forty-eight hour broth culture was made and 0.1 cc. quantities inoculated into tubes of litmus milk. Five tubes of the inoculated litmus milk were placed in a water bath adjusted well below 145°F. and held at this temperature for thirty minutes. At the end of the heating period the tubes were removed and cooled in tap water. The water bath was then adjusted 2° higher and five tubes heated for a like period. This procedure was repeated until a temperature well above that used in pasteurization had been reached. All tubes were incubated at 37°C. for approximately a week. Those tubes showing a red color due to acid production were recorded as positive, those having no color change as negative.

The acclimatizing process, or the second part, was accomplished by preparing a forty-eight hour broth culture and then inoculating 1 cc. quantities into each of 5 tubes of broth. These tubes were then immediately heated in a water bath at 142°F. for thirty

minutes, after which they were removed and cooled in tap water. One cubic-centimeter quantities were then taken from each tube and inoculated into five tubes each of broth and litmus milk. These tubes were then incubated at 37°.C for forty-eight hours. If growth occurred in the broth and the litmus milk tubes, 1 cc. quantities were taken from each of the broth tubes and reinoculated into 5 tubes of the same medium. These were then heated to 143°F. for thirty minutes and the same procedure repeated. This was continued until the culture was finally killed which

TABLE 1

A percentage comparison between the reduction of the total count and coli count of raw and pasteurized milk

TOTAL COUNTS		COLI COUNTS	
Percentage reduction	Number of samples	Percentage reduction	Number of samples
100–95 =	51	100–95 =	84
94–90 =	22	94–90 =	9
89–85 =	12	89–85 =	2
84–80 =	5	84–80 =	2
79–75 =	2	79–70 =	3
74–70 =	2		100
69–65 =	1	Average efficiency = 97.7 per cent	
64–60 =	2		
59–50 =	1		
49–35 =	1		
34–20 =	1		
	100		
Average efficiency = 91.4 per cent			

indicated that the absolute temperature may have been reached. The last set of broth tubes showing a positive growth were then used to inoculate other broth tubes. They were heated to the temperature that had previously proved fatal. If the cultures were killed after several trials at a given temperature, it was concluded that the absolute temperature of the strain had been reached.

DISCUSSION OF RESULTS

Table 1 shows the efficiency of pasteurization by comparing the percentages of reduction of the total counts and coli counts

before and after heating the milk. It is noted from the data that considerable reduction in both the total and the coli counts were obtained in a majority of the trials. When these reductions were averaged an efficiency of 91.4 per cent was obtained for the total count and 97.7 per cent for the coli count. Despite the

+ Growth
− No Growth
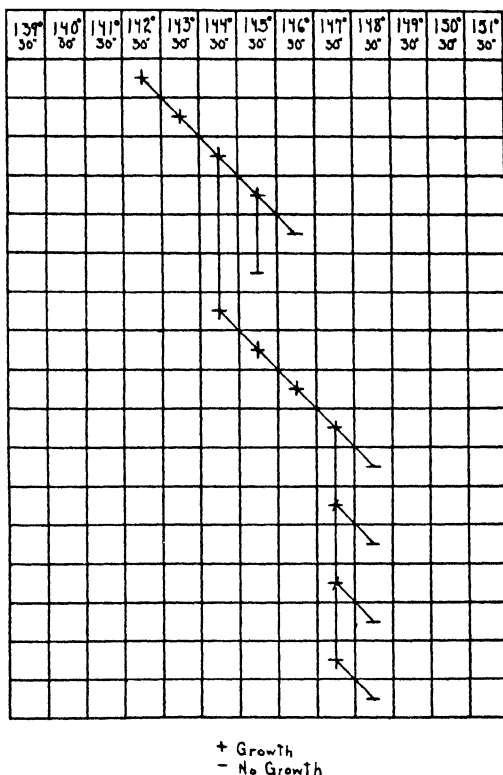
Fig. 1. Efforts to Raise Strain A Above Its Normal Thermal Death-Point of 144°F. for Thirty Minutes

fact that the pasteurization temperature should be sufficient to kill the organisms of the Escherichia-Aerobacter group, we note that a certain percentage of them survived. This occurred in 32 per cent of the number of samples examined.

The survival of Escherichia-Aerobacter organisms in pasteurized milk, as shown in table 1, may be influenced by acclimatiza-

tion. Figure 1 is a graph showing data obtained in raising a strain of *Escherichia coli* above its majority death-point of 144°F. By subculturing those organisms that resisted the various temperatures, it was possible to obtain a strain capable of resisting 147°F, a difference of 3°. These results do not necessarily indicate that this strain has actually acclimatized itself to the higher temperature as it was probably a survival of the fittest. The absolute temperature of a small percentage of the organisms was evidently higher than the majority temperature. This may have resulted in the survival of the most resistant ones. We should, therefore, expect the survival of those bacteria having an absolute death-point above the pasteurizing temperatures.

Reviewing the experimental evidence and the possible biological factors present in pasteurization, the statement can be made that organisms of the Escherichia-Aerobacter group may survive in milk that has been properly pasteurized. Therefore, the coli test cannot be used as a true index of proper pasteurization. This statement opposes the findings of Swenarton (9) and Jenkins (6), but confirms those of De Jong and De Graff (7), Ayres and Johnson (2), Shippen (8), and Hammer (5).

<div align="center">CONCLUSIONS</div>

1. The examination of 100 samples of pasteurized milk showed, in 32 per cent of the samples, that Escherichia-Aerobacter organisms were able to survive the temperatures used in commercial pasteurization.

2. The usual or majority death-point of a culture of *Escherichia coli* found to be 144°F. for thirty minutes, may have its absolute death-point of 145° for thirty minutes raised to 148°F. for thirty minutes. Inasmuch as this latter temperature is above the pasteurization temperature, the survival of members of the coli group may be expected.

3. The experimental evidence herewith presented indicates that the coli test is not a true index to proper pasteurization.

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MINERAL SUPPLEMENTS FOR DAIRY COWS[*]

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INTRODUCTION

This paper constitutes a final report on six and one-half years' investigation on the feeding of mineral supplements to dairy cows. Three progress reports[1] have already appeared and to these the reader is referred for our conclusions in detail up to the time these reports were published. The results thus far reported were obtained from a study of the feeding of tricalcium phosphate in the form of steamed bone meal specially prepared for animal feeding. In brief, the conclusion reached was that the benefit received from adding steamed bone meal to the ration of dairy cows under conditions existent in New England is very slight.

For the past two years the mineral supplement fed has consisted of a mixture of dicalcium phosphate and calcium carbonate in the respective proportions of four to one.

Köhler and Honcamp[2] have shown that lambs and goats assimilate dicalcic phosphate considerably better than they do tricalcic phosphate in the form of steamed or calcined bone. Having fed steamed bone to our cows for a considerable length of time with largely negative results, it was thought worth while to give the dicalcic phosphate a trial. The only variation from the experimental procedure described in our previous reports other than to substitute the dicalcic phosphate for the tricalcic has been

a further impoverishment of the ash content of the basal ration. This has been done by substituting for a portion of the hay larger amounts of dried apple pomace than were fed in the previous experiment.   The maximum amount of pomace fed to any one cow daily has been seven pounds.   According to Kellner's standard[3] the average deficiency of calcium in the basal ration has been about 31 per cent.   This is best illustrated by such a concrete example as appears below:

<p align="center">Cow "x" (average of the herd)</p>

	DAILY REQUIREMENT ACCORDING TO KELLNER'S STANDARD	
	Calcium	Phosphorus
	grams	*grams*
For maintenance (weight 1,115 pounds).....	36 2	11 1
For milk (34 pounds)	27 2	17 0
Total required	63 4	28.1
	DAILY INTAKE	
Average ration fed:		
Mixed hay, 17 pounds	37 0	16 2
Dried apple pomace, 5½ pounds....	3 8	2 8
Grain mixture, 11½ pounds	3 1	28 2
Total fed	43 9	47.2
Per cent excess or deficit	30.8−	68 0+
Add 8 ounces mineral mixture	61 7	29 9
	105 6	77.1
Per cent excess when mineral was fed	66 6%	174.4%

EXPERIMENTAL METHODS

At the commencement of the experiment the herd consisted of 10 milking Holstein cows, 2 milking Jersey cows, 3 Holstein heifers and 1 Jersey heifer.   At the conclusion, due to unavoidable changes, the herd was made up of 10 milking Holsteins and

[3] Kellner's standard calls for a minimum of 32.5 grams of calcium (Ca) and 10 grams of phosphorus (P) daily for maintenance of a 1000-pound cow, and in addition 0.8 gram of calcium and 0.5 gram of phosphorus daily for each pound of milk produced.

3 milking Jerseys, but no heifers.   The animals were well housed and received good care, and excepting in bad weather were turned daily into adjacent sheltered yards for air, exercise and sunlight.   The cows were barn fed during the entire year; the young stock were turned to pasture from approximately May 20 to October 15.

The entire herd was fed on a basal ration of first cut hay, dried apple pomace and a grain mixture during the entire period of observation, excepting during the summer months, when, in addition, non-leguminous green material in the form of oats, millet and corn were fed in amounts not exceeding 25 pounds daily to any one animal.

The average amount of hay fed daily to the milking cows was 17 pounds; dried apple pomace, $5\frac{1}{2}$ pounds; grain mixture, $11\frac{1}{2}$ pounds; and green feeds, 23 pounds.   Green feed (non-leguminous) was fed for a period of about 3 months each season (July 1 to October 1).   When fed it was substituted for a portion of the hay in the proportion of 5 pounds of green feed to 1 pound of hay, thus reducing the average daily ration of hay for those months to approximately 12 pounds.   Salt was added to the extent of $\frac{3}{4}$ pound to each 100 pounds of the grain mixture, and lump salt was always available in boxes in the out-of-door sheds.   In addition the so-called mineral cows received daily, mixed with the grain, an average of 8 ounces of the dicalcium phosphate-calcium carbonate mixture.   The amount of calcium carbonate fed (1 part in 5 of the mixture) was added to maintain the same ratio between calcium and phosphorus as existed when the tricalcic phosphate was fed.   Running water was before the animals at all times.   The cows were fed and milked twice daily.   The hay, which was grown on the station grounds, was composed of mixed grasses, timothy usually predominating, and generally was of good quality.   It averaged 7.58 per cent protein, 0.48 per cent calcium and 0.21 per cent phosphorus.   The dried apple pomace contained 5.29 per cent protein, 0.15 per cent calcium and 0.11 per cent phosphorus, while the grain mixture, consisting of 30 per cent ground oats, 15 per cent Diamond gluten meal, 15 per cent cottonseed meal, 20 per cent wheat bran and 20 per cent

corn meal, averaged about 17 per cent digestible protein, 45 per cent digestible carbohydrates, 0.06 per cent calcium, and 0.54 per cent phosphorus.

The apple pomace was weighed into wooden trays daily, moistened, and the grain mixed with it before being fed. The hay and green forage were weighed daily into 4-bushel baskets.

Composite samples of all feeds were taken from each lot purchased. A five-day composite sample of the milk of each cow was taken monthly. All feeds and milk were submitted to chemical analyses.

PRESENTATION AND DISCUSSION OF RESULTS

The effect of the supplement has been judged by the same criteria as those used in measuring the effect of tricalcium phosphate, viz: (1) general appearance and body weight of the cows; (2) growth of young cows and heifers; (3) milk yield; (4) composition of the milk, especially its ash content; (5) reproduction.

General appearance

As in all our work on mineral supplements, the general condition of the cows has been followed closely, observations being recorded from month to month. With the exception of one individual the cows in both groups have maintained themselves well. A study of the detailed record of their condition at the commencement of the experiment and again at the close shows that the cows receiving the supplement had a slight advantage in this respect (see table 1).

Body weight

Respecting maintenance of body weight there are six individuals that were mature when the experiment was commenced and which consequently can be considered from this standpoint. The stage of gestation they were in at the beginning having been noted, the date during the present season when they were at a similar stage has been ascertained and the average of two weighings on consecutive days as near that date as possible has been taken (see table 2).

The mature cows receiving the supplement made a considerable gain in weight during the two years of the experiment, while the other group showed little change. The supplement may have made this difference, but with this number of animals it is unsafe to draw a positive conclusion.

TABLE 1

Showing condition of cows

COW NUMBER	AGE JULY 1, 1928	BREED	GENERAL CONDITION	
			July 1, 1926	July 1, 1928
Non-mineral group				
6	11	Grade Holstein	Good	Fair
10	13*	Grade Holstein	Fair	Good*
15	9	Grade Holstein	Very good	Good
35	7	Grade Holstein	Good	Good
42	5†	Grade Holstein	Excellent	Good†
43	6	Purebred Jersey	Good	Excellent
88	3	Grade Holstein	Excellent	Very good
99	3	Grade Holstein	Fair	Fair
Mineral group				
11	13*	Grade Holstein	Good	Fair*
12	11	Grade Holstein	Excellent	Excellent
22	8	Grade Jersey	Fair	Good
33	7	Grade Holstein	Good	Excellent
40	6	Grade Holstein	Good	Good
71	4	Grade Holstein	Fair	Good
98	3	Grade Holstein	Good	Fair
100	3	Grade Jersey	Good	Excellent

* When sold August 8, 1927.
† When slaughtered January 10, 1928.

Growth of young cows and heifers

These animals all received a basal ration low in mineral matter from the time they were weaned at four to five months of age. In addition the "mineral" group received steamed bone meal until the beginning of the present experiment when the dicalcium phosphate-calcium carbonate mixture was substituted for the bone meal.

Ten of the individuals were immature at the commencement of the experiment, six of them being young cows (under five years of age), and four were unbred heifers. In the case of the young cows variations due to variable stage in gestation have been taken into account as with the mature cows. With the heifers unbred when the experiment started obviously this method could not be used, but three of the four are in an almost identical stage of gestation this season and·the fourth is also bred, though not

TABLE 2

Maintenance of body weight in the mature cows

COW NUMBER	WEIGHT ON JULY 1, 1926	WEIGHT ON A COMPARABLE DATE THIS SEASON (1928)	GAIN OR LOSS
Non-mineral group			
6	1,270	1,300	+30
10	1,205	1,170*	−35
15	1,315	1,345	+30
Net gain or loss for the group			+25
Mineral group			
11	1,275	1,300*	+25
12	1,405	1,510	+105
22	700	820	+120
Net gain or loss for the group.			+250

* Weight when sold in August, 1927.

as far advanced, so from that standpoint their weights can be considered as quite comparable (see table 3).

The presence of one Jersey in each age class (no. 43 in the young cow class, no. 100 in the heifer class) somewhat complicates analysis of these weight data. A truer picture of the case is obtained if the growth increments are presented on a percentage basis, as in the last column of table 3.

The above weights indicate a considerable difference in favor of the "mineral" group, but it is discounted by the fact that they average six months younger in the case of the young cows and

two months younger in the case of the heifers than the "non-mineral" group. Unfortunately the weights of the groups at identical ages are not comparable due to considerable differences in stage of gestation. If nos. 43 and 71 be dropped out of the

TABLE 2

Growth of young cows and heifers

	WEIGHT ON JULY 1, 1926	WEIGHT ON A COMPARABLE DATE THIS SEASON (1928)	GAIN	
Young cows				
	pounds	*pounds*	*pounds*	*per cent*
Non-mineral group:				
Cow 35.........................	910	1,010	100	11.0
Cow 42.........................	1,260	1,350*	90	7.1
Cow 43.........................	830	850	20	2.4
Net gain for the group.........			210	
Mineral group:				
Cow 33.........................	1,215	1,355	140	11.5
Cow 40.........................	900	980	80	8.9
Cow 71.........................	955	1,345	390	40.8
Net gain for the group.........			610	
Heifers				
		WEIGHT ON JULY 1, 1928		
Non-mineral group:				
Heifer 88......................	685	1,135†	450	65.7
Heifer 99......................	570	1,140	570	100.0
Total gain....................			1,020	
Mineral group:				
Heifer 98......................	575	1,130	555	96.5
Heifer 100....................	445	855	410	92.1
Total gain....................			965	

* Weight in October, 1927, no later weight available—cow in isolation stall.

† In a somewhat earlier stage of gestation than the other three heifers.

young cow group the others are of identical age, and the difference is seen to be very small, 9.05 per cent increase in the non-mineral group as against 10.2 per cent in the "mineral" group. Among the heifers if no. 88 were of the same age as the other three (four

months younger than she actually is) her rate of increase in weight would in all probability be enough higher to offset the difference of 11.4 per cent actually found. All things considered the growth of the young animals was probably not materially influenced by the addition of the mineral supplement.

Another angle from which growth records have been viewed is that of weight of the young stock born and reared during the course of the experiment and now in the herd, as compared with the weight of their dams at a similar age. Of the ten individuals listed in table 3 two are as large as their dams were at the same age, six are larger, and two are smaller. The grouping in the experiment is as follows:

Mineral supplement		*No mineral supplement*	
Larger than dam	 3	Larger than dam	. . . 3
Same size	 1	Same size	 1
Smaller	 1	Smaller. 1

All are Holsteins and sired by the same bull, except the two which are the same size as their dams, which are Jerseys. These facts dispose of possible variation due to influence of different sires, and the identical make-up of the two groups is further evidence that the mineral supplement had little, if any, effect on growth.

Photographs of all cows in the herd at the conclusion of the experiment appear in figures 1 and 2.

Milk yield

The average production of the herd has been well maintained all through the experiment. The corrected[4] daily yield per cow for the whole herd has been as follows:

	Milk pounds	Total solids pounds	Fat pounds
First year (10 cows)	26 47	3.32	1 07
Second year (11 cows).....	27 59	3 46	1 09

[4] Corrections for differences in length of lactation and stage of gestation have been made according to the method of Gaines and Davidson, described in Illinois Agr Expt Sta. Bulletin No. 272 Corrections for age have not been necessary as the groups were of approximately the same age, and the age of the herd as a whole did not increase, as some older cows were sold and heifers took their places Corrections for breed are taken care of by including in the corrected summary the total solids and fat produced as well as the total milk yield.
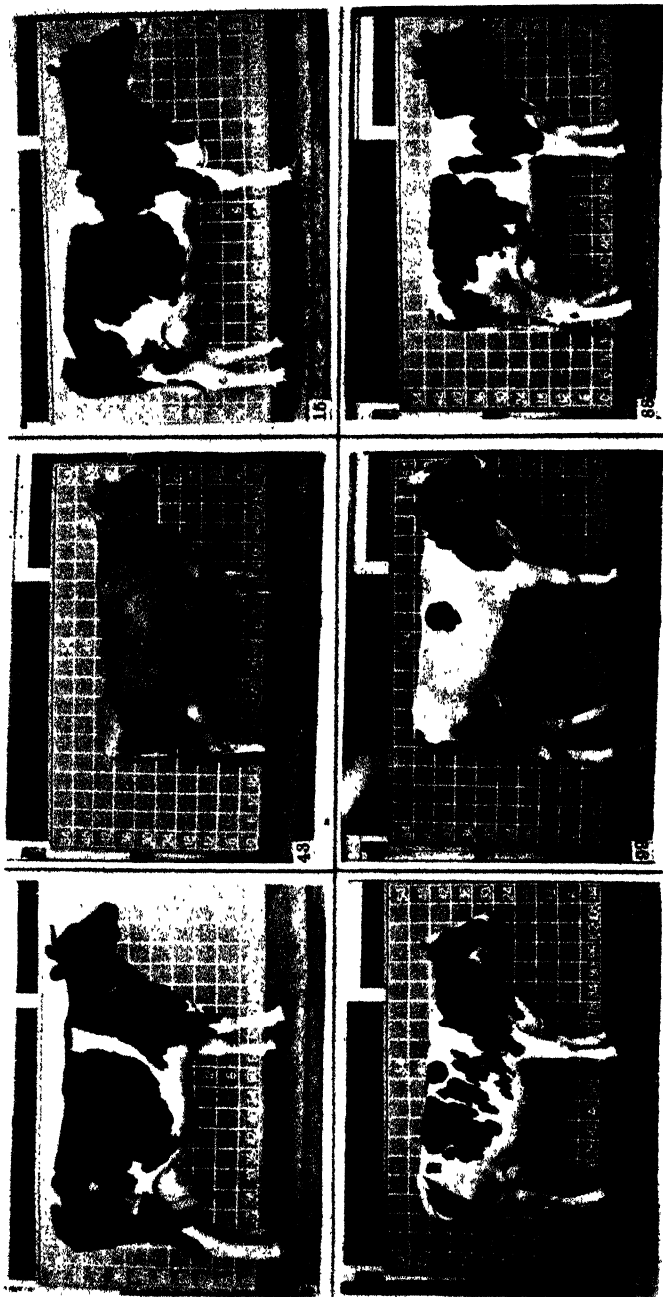
FIG. 1 COWS TYPICAL OF THOSE FED NO MINERALS; TAKEN AT THE CONCLUSION OF THE EXPERIMENT
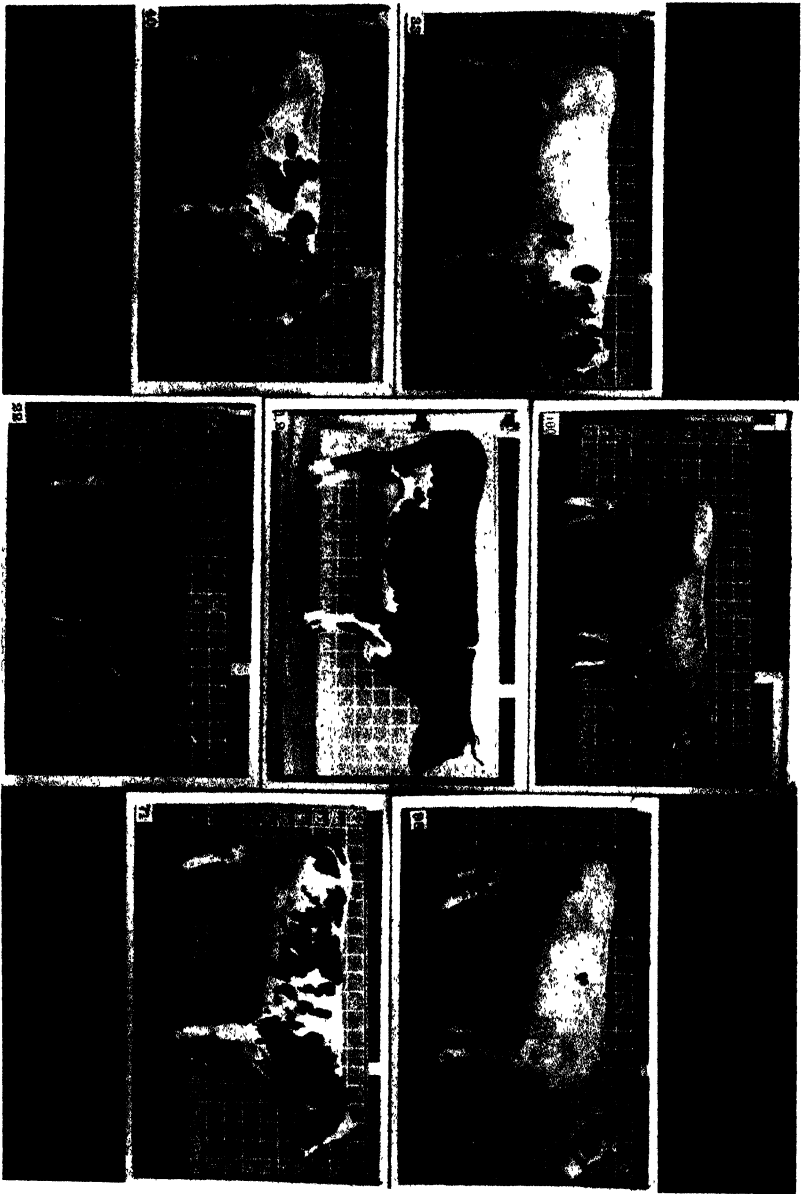
FIG 2 Cows Typical of Those Fed Minerals; Taken at the Conclusion of the Experiment

The corrected daily yield per cow for the two groups during the entire experiment and year by year appears below:

Entire experiment:	Milk *pounds*	Total solids *pounds*	Fat *pounds*
"Mineral" group (10 cow years)	25 09	3 29	1 04
"Non-mineral" group (11 cow years)	28 85	3 58	1 14
Year by Year:			
"Mineral" group (first year, 5 cows).	25 03	3 14	1 00
"Mineral" group (second year, 5 cows)	25 38	3 25	1 02
"Non-mineral" group (first year, 5 cows) .	27 92	3 49	1 14
"Non-mineral" group (second year, 6 cows)..	29 64	3 65	1 15

TABLE 4

Composition of the milk[*][†]

	WHOLE HERD	MINERAL GROUP	NON-MINERAL GROUP
Total solids .	12 53	12 58	12 53
Fat.	3 99	3 96	4 03
Total ash .	0 711	0 712	0 710
Ash as per cent of total solids .	5 67	5 66	5 66
Calcium .	0 121	0 123	0 120
Phosphorus	0 099	0 102	0 097

* Expressed as percentage of the liquid milk

† These figures have been corrected for differences in the breed make-up of the groups   Corrections for stage of lactation have not been necessary, as the average stage of lactation at the time the samples were taken was the same in both groups.

The "non-mineral" group was somewhat superior to the "mineral" group in production but it would be unwise to conclude from this that the mineral supplement had a detrimental effect in this respect.   The probability is that the difference is due to variations in the inherent capacity of the individuals in the two groups to produce milk.   An endeavor has always been made to keep the groups as evenly matched in this respect as possible, but due to the impossibility of predicting in advance the performance of a cow, or more especially of a heifer, in any given year, this is a matter which in practice is difficult to regulate. That the endeavor in this particular instance was not successful is evidenced by the figures.

The conclusions that seem justified are two: (1) that the low ash ration apparently had no immediate adverse effect on production; (2) that the mineral supplement apparently had no immediate favorable effect.

Composition of the milk

The milk from each cow in the herd is sampled for five consecutive days each full month that she is in milk. Total solids and fat have been determined each month during the course of the experiment. Total ash, calcium and phosphorus have been determined every three months.

The differences between the groups in this respect are seen to be very slight, and while they favor the mineral group (except in the fat content) they are not of sufficient magnitude to be of any significance, as shown by table 4.

Reproduction

Reproductive troubles have not been serious in either group. The average length of time after calving before reappearance of oestrum has been:

	days
Mineral group	38
Non-mineral group	45

Regarding regularity in recurrence of oestrum the records show the following:

	Good	Fair
Mineral group	11	1
Non-mineral group	9	3

The average number of services required the breed has been:

	times
Mineral group	1.13
Non-mineral group	1.30

Seventy-one per cent of the breedings have been from one service, only one cow in each group giving serious trouble in this respect.

Other irregularities have been:

One premature birth (eight months) in the mineral group.

One case of *nymphomania* (chronic buller) in the mineral group.

One case of *cervicitis* (inflammation of the neck of the uterus) in each group.

Three cases of retained afterbirth (two of them in the same cow in successive years) in the non-mineral group.

One calf died *in utero* at about the sixth or seventh month stage as a result of cervical and general uterine infection in the dam. This was in the non-mineral group.

Apparently the cows that received the mineral supplement were a little closer to normal in their reproductive function than those that did not receive it. They came in heat a little sooner after calving, recurrence of heat was slightly more regular, and they bred a little more readily. Other irregularities are about evenly divided between the two groups. In any case the reproductive troubles have not been greater than are ordinarily encountered in practical herd management.

The condition of the calves at birth and subsequently is also of interest in this connection. Twenty-six calves have been dropped in the herd during the course of the experiment. Classification of these according to group and condition appears below.

	Vigorous	Good	Fair	Delicate
Mineral group..	3	9	1	1
Non-mineral group..................... ...	3	5	4	0

The main difference here is seen to be between the "good" and "fair" calves. The statistics undoubtedly favor the mineral group but they need to be examined as to make-up of the groups with respect to sex and breed, as these determine to a considerable extent the apparent vigor of a calf. Bulls are usually more lusty than heifers; Holsteins are generally more rugged than Jerseys.

Of the four "fair" calves in the non-mineral group three were heifers, and the fourth although a bull was a Jersey. On the other hand, of the nine "good" calves in the mineral group, six were heifers, and three of these heifers were Jerseys. These situations just about offset each other so that the balance is still somewhat in favor of the mineral group. It would seem that the cows receiving the mineral supplement produced on the whole somewhat better calves. It must be stated, however, that some

very fine calves have come from the cows that have not received the supplement.

SUMMARY AND CONCLUSIONS

The object of this work has been to determine the value for dairy cows of a mineral supplement consisting of 80 per cent dicalcic phosphate and 20 per cent carbonate of lime added to a ration supposedly deficient in lime.   Previous work with tricalcic phosphate in the form of steamed bone meal as a source of lime and phosphorus showed little, if any, advantage in supplying these elements in that form.   As dicalcic phosphate is somewhat more soluble and had given better results elsewhere with lambs and goats that the tricalcic phosphate did, it was thought worthy of a trial with cows.

A special effort was made to have the basal ration of the cows low in lime.   To this basal ration there was added for half of the herd (known as the "mineral" group) sufficient of the phosphate-carbonate mixture to theoretically make good the deficiency. The experiment was carried on for two years with the following results:

1. With one exception all the cows in the herd maintained their general condition well.   The mature cows that received the mineral supplement maintained themselves slightly better in this respect than did those not receiving the supplement.   They also made a considerable gain in weight while the non-mineral group showed little change.

2. The supplement had little, if any, effect on the growth of the young cows and heifers.

3. The low ash ration apparently had no immediate adverse effect on milk production and the mineral supplement apparently had no immediate favorable effect.

4. The composition of the milk was not significantly affected.

5. Reproductive troubles were not serious in either group but the cows receiving the mineral supplement were a little nearer normal and produced on the whole somewhat better calves than did those that did not receive it.

6. It must be emphasized that none of the differences between

the groups were sufficiently striking to warrant as a general recommendation the use of the dicalcic phosphate-calcium carbonate mixture.   Where cows are average producers (5000 to 8000 pounds) and where they are fed normally on good quality roughage the need of a mineral supplement is not indicated.   For heavy producers (10,000 and upwards) it is probably good insurance to supply supplemental lime and phosphorus, but the efficacy of such a practice is by no means well established.   It has been nothing short of amazing to note the persistency with which the herd of cows devoted to this study have maintained their milk production and for the most part their own well-being on low ash rations over a period of years.   If these cows which average over 9000 pounds of milk yearly, several of them being eleven to twelve thousand pound cows, can make such a showing on abnormal rations, it seems reasonable to infer that the average New England cow on good quality roughage must have a considerable margin of safety as far as minerals are concerned.

This paper is presented with full realization of the beneficial effects reported by numerous other investigators who have fed mineral supplements and without prejudice to their results.   Our negative results are attributed in the case of calcium to a considerably higher content of this element in the hays fed than has been found in similar hays elsewhere (0.4 to 0.5 per cent as contrasted with 0.2 to 0.3 per cent), and in the case of phosphorus to quite liberal grain feeding.   This latter is normal for New England, and analyses of a large number of Massachusetts hays in addition to those we have fed shows an average calcium content of 0.41 per cent.

It is our opinion that the problem of mineral deficiency in rations is largely a regional one, and that where the roughage carries a reasonably high percentage of calcium and liberal grain feeding is practiced mineral supplements will not be necessary for the average cow.

Regarding Kellner's standard it would seem with respect to calcium that his minimum is well above the danger line, and with respect to phosphorus that where cows are grain fed the amounts of this element they receive are well above the minimum he prescribes.

THE INFLUENCE OF MOLASSES AND SODIUM CHLORIDE IN DAIRY RATIONS ON THE LACTOSE AND CHLORIDE CONTENT AND TASTE OF MILK*

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The importance of taste of dairy products is being emphasized more and more by dairy research workers, commercial distributors of dairy products and by dairy control officers. A knowledge of the factors that influence taste is necessary before progress can be made in improving taste.

Roadhouse and Koestler (1), working with milk at the Dairy and Bacteriology Experiment Station, Liebefeld, Switzerland, found that the chlorine-lactose number[1] of milk as described by Koestler (2) was the greatest single factor in determining the primary taste of the milk. Normal milk that was relatively high in lactose and relatively low in chloride content usually gave a sweet pleasing taste, while milk low in lactose and high in chloride generally gave an unpleasant and often an astringent or salty taste.

In the present experiment an attempt was made to change the lactose-chloride relation of the milk by changing the rations of dairy cows and thus change the taste of the milk.

Morgen, Berger and Fingerling (3) have reported that the concentration of lactose in the milk was not significantly affected by any of the changes in rations which they used. In the present study the maximum amount of carbohydrate, in the form of cane molasses, and the maximum sodium chloride which the cow would consume were fed.

[1] The value of the chlorine-lactose number is found by multiplying by 100 the ratio of percentage chloride to percentage lactose.

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PROCEDURE

Four cows producing milk with a pleasing taste were selected from the Station herd. They were fed a special salt-free basal ration to which no salt was added. No feed was eaten by the animals during the five-hour period previous to milking.

The animals were kept on the basal ration for 26 days and composite samples of milk were collected from twenty-four-hour samples on two consecutive days each week. The samples were examined for taste and for lactose and chloride content. The lactose determinations were made gravimetrically, according to official methods, using Soxhlet and Wein's (4) tables and the chloride determinations by a modification of Volhard's (5) method.

After the preliminary feeding period the same basal ration was continued, and in addition molasses and sodium chloride were given to certain cows for forty-three days, according to the following plan.

Cow 226: Control animal; received the basal ration (without salt).

Cow 404: Received basal ration with a daily average of 2.5 ounces of salt.

Cow 126: Received basal ration with a daily average of 5 pounds of molasses and no salt.

Cow 393: Received basal ration with a daily average of 3.6 ounces of salt and 6 pounds of molasses.

The basal ration consisted of the following:

	Parts
Rolled Barley	4
Oats	2
Wheat bran	2
Linseed meal	1

The sampling and analyses were continued as during the preliminary period. All the cows were then returned to the basal ration for 20 days and the milk sampled and analyzed as previously.

The tables shown in this paper include the averages of the weights of milk, analyses and taste scores of the two consecutive twenty-four-hour samples collected each week during the course of the experiment.

TABLE 1

Cow 226. Lactose and chloride content and taste score of milk from control cow receiving basal ration (without salt)

	DATE	MILK 24 HOURS	TASTE SCORE	PER CENT LACTOSE	CHLORIDE
	1929	*pounds*			*grams per liter*
Preliminary period............	March 8	32.7	13.52	4 82	0.83
	March 12	31.7	13.48	4 81	0 87
	March 20	32.3	13.54	4.76	0.93
	March 26	34.0	13 50	4.78	0.96
	April 2	33.7	13.32	4.69	0.97
Experimental period..........	April 10	31 8	13.27	4.81	0 95
	April 16	32.9	13 31	4.67	0 96
	April 24	35 1	13.34	4 71	0.94
	May 1	33 7	13 31	4.73	0.94
	May 8	32.5	13.39	4 74	0.97
	May 15	32.9	13.31	4.63	0.99
Post-experimental period	May 22	32 3	13 35	4 66	1 00
	May 29	28.3	13 28	4 72	1.08
	June 4	27 9	13 45	4.74	1 04

TABLE 2

Cow 404. Lactose and chloride content and taste score of milk from cow receiving basal ration and sodium chloride

	DATE	MILK 24 HOURS	TASTE SCORE	PER CENT LACTOSE	CHLORIDE
	1929	*pounds*			*grams per liter*
Preliminary period............	March 8	37.7	13 57	4.84	0.77
	March 12	34.2	13 56	5 09	0 71
	March 17	34.5	13.55	5.03	0 71
	March 20	33 1	13.47	5.06	0.76
	March 26	32 5	13 48	4.93	0.79
	April 2	32 1	13.52	5.03	0.72
Experimental period..........	April 10	29 1	13 16	5.00	0 82
	April 17	29 8	13.46	4.74	0 85
	April 24	28.7	13.42	4.64	0 90
	May 1	29.0	13.44	4.73	0 82
	May 8	26 2	13.27	4.86	0.81
	May 15	27.7	13 38	4.71	0.83
Post-experimental period.......	May 22	28.1	13 40	4.76	0 84
	May 29	28.4	13.38	4.84	0.84
	June 4	27 6	13.50	4.80	0.85

TABLE 3

Cow 126. Lactose and chloride content and taste score of milk from cow receiving basal ration and molasses

	DATE	MILK 24 HOURS	TASTE SCORE	PER CENT LACTOSE	CHLORIDE
	1929	*pounds*			*grams per liter*
Preliminary period	March 8	19.5	13 36	4.50	1.24
	March 12	20 5	13 37	4.45	1.32
	March 20	19 3	13 38	4 47	1 34
	March 26	21.1	13 34	4 46	1.34
	April 2	20.3	13 38	4 42	1 35
Experimental period	April 10	20.2	13.40	4 43	1 36
	April 16	20.2	13 38	4 35	1 42
	April 24	21.1	13 16	4 34	1.40
	May 1	20.1	13 25	4 37	1.42
	May 8	18 8	13 32	4 44	1 37
	May 15	17.9	13.23	4.40	1.39
Post-experimental period.......	May 22	17.2	13 38	4 47	1 39
	May 29	14 5	13.35	4 54	1 44
	June 4	14.1	13 35	4.48	1 43

TABLE 4

Cow 393. Lactose and chloride content and taste score of milk from cow receiving basal ration with molasses and sodium chloride

	DATE	MILK 24 HOURS	TASTE SCORE	PER CENT LACTOSE	CHLORIDE
	1929	*pounds*			*grams per liter*
Preliminary p riod............	March 8	24 6	13 48	4 42	0 90
	March 12	26.3	13 42	4 45	0 90
	March 20	24 5	13 45	4.49	0 92
	March 26	25.8	13.43	4 42	0 94
	April 2	25.6	13.46	4.34	0 97
Experimental period...........	April 10	20 9	13 49	4.34	0 97
	April 16	21.5	13.48	4.29	1.01
	April 24	23 7	13 47	4.13	0 99
	May 1	22.8	13.47	4.16	1 02
	May 8	20.5	13.45	4 22	1.00
	May 16	20.9	13.42	4.13	1.07
Post-experimental period.......	May 22	20.0	13.32	4.18	1.10
	May 29	17.5	13.38	4.32	1.13
	June 4	14.4	13.38	4.28	1.08

DATA

The results secured from the feeding, sampling and analyses of each animal are shown in tables 1 to 5, inclusive.

The summary of the data presented in tables 1 to 4 is shown in table 5 and is an average of total analyses for the preliminary, experimental and post-experimental periods.

TABLE 5

The average lactose and chloride contents and taste scores of the milk of each cow during the periods of the experiment

	COW	PRELIMINARY PERIOD	EXPERIMENTAL PERIOD	POST-EXPERIMENTAL PERIOD
Per cent lactose	226	4.77	4.72	4 71
	404	5.00	4.78	4 80
	126	4 46	4.39	4.50
	393	4 42	4.21	4.26
Chloride, grams per liter	226	0.91	0.96	1 04
	404	0.74	0.84	0.84
	126	1.32	1.39	1.42
	393	0.93	1.01	1.10
Taste scores	226	13.51	13.32	13.36
	404	13 53	13 37	13 43
	126	13.37	13 29	13 36
	393	13.45	13.46	13.36

DISCUSSION OF RESULTS

Cow 226, the control, showed a very slight average decrease of 0.06 per cent lactose during the entire period, which is considered normal with the advance in the period of lactation. The chloride increased 0.13 gram per liter during the entire period, which also can be considered normal with the advance of lactation. The taste score approximately paralleled the lactose content.

Cow 404, receiving an average of 2.5 ounces of sodium chloride daily after the preliminary period, produced milk with a very slight average decrease of 0.22 per cent lactose during the experimental period, and 0.2 per cent during the post-experimental period.

The chloride content increased an average of 0.1 gram per liter during the experimental period, which is considered normal with the advance in lactation. It is indicated from these results that the chloride was not abnormally increased in the milk with the increase of chloride in the ration.

The taste score decreased an average of 0.16 of a point during the experimental period, and corresponds closely with the lowering of the lactose content during the same period.

Cow 126, receiving an average of 5 pounds of molasses daily, after the preliminary period produced milk containing an average of 0.07 per cent less lactose during the feeding of the molasses than during the preliminary period, and 0.11 per cent less during the post-experimental period. It is evident from these results that the feeding of the molasses did not increase the lactose content during the experimental feeding.

The chloride content increased 0.1 gram per liter during the experimental period, which is considered normal with the advance in lactation.

The taste score averaged 0.08 of a point lower during the experimental period, which would be expected to accompany the lower lactose content.

Cow 393, receiving an average of 6 pounds of molasses and 3.6 ounces of salt daily after the preliminary period, produced milk containing 0.21 per cent less lactose during the experimental period than during the preliminary period, and 0.05 per cent less than during the post-experimental period. The results indicate that the molasses also fed to this cow did not increase the lactose content during the experimental feeding.

The chloride content increased slightly and at the same rate during the preliminary, experimental and post-experimental periods, and at approximately the same rate as the control cow. This increase in chloride content would be expected during the advance of lactation.

The taste score remained constant during the preliminary and experimental periods, with a slight decrease of an average of 0.1 point during the post-experimental period.

The results indicate that the feeding of molasses and sodium

chloride did not increase the lactose or abnormally increase the chloride content in the milk.

CONCLUSIONS

1. The addition of molasses in the amount of 5 to 6 pounds daily to a basal ration of grain and alfalfa hay, fed to two cows, did not increase the lactose content of the milk.

2. The addition of sodium chloride in the amount of 2.5 to 3.6 ounces daily to a basal ration of grain and alfalfa hay, fed to two cows, did not increase the chloride content of the milk abnormally.

3. In general, the taste score of the milk followed the trend of the lactose content; the taste score lowering and rising as the lactose per cent decreased and increased.   It is also to be noted that there was a correlation between the lowering of the taste score and lactose percentage and an increase in the chloride content.

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FACTORS INFLUENCING THE PROPERTIES OF FERMENTED RECONSTRUCTED MILK*

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INTRODUCTION

The limited supply of normal skim milk suitable for fermented milk manufacture, especially in the large centers of population, together with the steadily growing demand for fermented milks, has caused the plant operators to search for a substitute for normal skim milk.

This paper presents the results of a study of the influence of the manufacturing procedure and of the quality of starter used, on the fermented milk made from reconstructed skim milk. The processes studied are those that are subject to considerable variations between plants, or in the same plant.

REVIEW OF LITERATURE

Burke (1) defined the qualities of a good grade of Commercial Buttermilk.

Larsen and White (2) found that a good quality starter can be made by using reconstructed skim milk as a substitute for normal skim milk. They further showed the danger of using too much powder in the manufacture of the reconstructed milk.

Dahle and Palmer (3) in an investigation of the properties of reconstructed milks made a milk which gave a slightly lower soluble protein content, a slightly lower buffer action at low concentrations of alkali and acid, had a slightly higher viscosity than normal milk, but closely resembled normal milk in freezing point, specific gravity, and specific conductivity.

Hammer (4) recommended an incubation temperature of from 21 to 23°C. (70° to 74°F.) for the commercial cultures.

Knaysi (5) studied fermented normal milk and found that an increase in the amount of inoculum gave an increase in titratable acidity, viscosity, firmness of the curd, and an increased tendency to whey off.

Hammer (6) and Knaysi (5) concur in their findings that an increase in the pasteurization exposures increases the firmness of the curd of fermented normal milk.

Hammer and Baker (7) found that lowering the pasteurization exposures lowered the rate of acidity development and lengthened the time of coagulation.

Yaxis (8), Messcher (9), and Burke (10), and Reid (11) agree that the addition of small amounts of gelatin to normal skim milk before fermentation will result in an increased viscosity and prevents wheying off of the fermented milk.

PROCEDURE

Studies were made of the effect of the following factors on the fermented milk made from reconstructed skim milk:

1. The quantity of inoculum.
2. The process of cooling after incubation.
3. The pasteurization exposure.
4. The addition of different amounts of gelatin.
5. The addition of normal skim milk.
6. The cooling of fermented milk before breaking the curd.
7. A comparison of the qualities of fermented milks made from gelatinated reconstructed skim milk, normal skim milk, and reconstructed skim milk.

Reconstructed skim milk was made for these studies by the addition of water to skim milk powder in the ratio of 9 pounds of water to 1 pound of skim milk powder. The skim milk powder used for these studies was selected by giving careful consideration to its aroma, flavor, solubility and acidity.

The milk so made was then pasteurized at 180°F. for thirty minutes, cooled to 72°F., transferred into sterile quart bottles, and inoculated.

The inoculated milk was then incubated at a temperature of

72°F. for a period of from twelve to fourteen hours and then removed to a cooling room, the temperature of which was maintained at 42°F., for a period of eight hours.

After cooling the curd was broken by gently agitating the bottles, each bottle receiving a like amount of agitation.

Three samples were then transferred into sterile bottles for testing and scoring at the end of twenty-four, seventy-two, and one hundred and twenty hours storage at 42°F. The remainder of each lot was used for immediate testing and scoring.

The aroma and flavor of each lot were scored on the basis of 100 points and a score of 75 for aroma and flavor was deemed palatable. The body and texture were scored on the basis of 100 points and a score of 85 was deemed necessary to be considered a good quality of fermented milk. The acidity was determined by titration against tenth normal sodium hydroxide, and calculated as per cent lactic acid. The viscosity time was determined by the time in seconds required to empty a 50 cc. pipette to a mark 1 inch below the bulb. The whey formation, the appearance of the curd, and the presence of gas were also noted.

EXPERIMENTAL DATA

The inoculation of reconstructed milk with various percentages of starter

Six series, of 5 lots each, were made to determine the effect of varying the amount of starter used in the inoculation.

Table 1 shows the effect of inoculating the reconstructed milk with increasing amounts of starter. Increasing the increment of inoculum increased the score of the aroma, flavor, body, texture, percentage of acidity, and the viscosity in the fermented milks, at the time of breaking the curd. The increase in the score of the aroma and flavor was largely the result of a submerging of powder taste and odor due to the increased acidity. The increase in body and texture score was due to the development of a stronger, more viscous curd, all lots showing a consistent smoothness.

The appearance of the respective lots was not affected and no wheying off occurred in the fresh buttermilk.

Storage for twenty-four hours resulted in a decrease in the score of the aroma, flavor, body, and texture, in titratable acidity, and in viscosity time, followed by a slight increase at the end of seventy-two hours storage.   Continued storage at the same tem-

TABLE 1

The effect on the properties of fermented reconstructed milk when different percentages of starter were added

NUMBER OF LOT	INOCULUM ADDED	SCORE OF AROMA-FLAVOR	SCORE OF BODY-TEXTURE	ACIDITY	VISCOSITY TIME
	per cent	*per cent*	*per cent*	*per cent*	*seconds*
I	2	75 60	79 00	0.789	59
II	4	77 80	80 50	0 807	72
III	6	78 80	83 00	0 853	72
IV	8	78 80	84 00	0 855	77
V	10	79 60	84 30	0.863	78

TABLE 2

The effect on the whey formation of fermented reconstructed milk when different percentages of starter were added

NUMBER OF LOT	INOCULUM ADDED	SAMPLES WHEYING OFF AND AMOUNT OF WHEY FORMED ON STORAGE					
		24 hours		72 hours		120 hours	
		$\frac{1}{4}-\frac{1}{2}$ inches	$\frac{1}{2}-\frac{3}{4}$ inches	$\frac{1}{4}-\frac{1}{2}$ inches	$\frac{1}{2}-\frac{3}{4}$ inches	$\frac{1}{4}-\frac{1}{2}$ inches	$\frac{1}{2}-\frac{3}{4}$ inches
	per cent						
I	2	1	0	2	0	2	0
II	4	1	1	2	1	2	1
III	6	0	2	0	1	0	1
IV	8	0	1	0	0	0	1
V	10	0	2	0	0	0	1

perature caused a downward trend in the numerical values of the properties at the end of one hundred and twenty hours.

Table 2 shows the whey formation of the fermented milk.   The results show a tendency for lots I and II to whey off on storage. A slight amount of whey, however, was not considered harmful to the product.

The appearance of the samples, excepting those showing considerable whey formation, was very uniform.

The effect of the cooling process after pasteurization on the properties of fermented reconstructed milk

Six series, of 4 lots each, were made to determine the effect of the cooling process on the properties of the fermented reconstructed milk.

Cooling the heated milk over a surface cooler tends to give a product somewhat less desirable in flavor and aroma as shown in table 3, and is thought to be due to the aeration of the heated product. Quick cooling does not result in any marked improvement in the properties of the reconstructed milk because of the danger of contamination due to the exposure to air. Variations occurring in the numerical value of the other properties are insig-

TABLE 3

The effect of the cooling process after pasteurization on the properties of fermented reconstructed milk

| NUMBER OF LOT | COOLING PROCESS | | SCORE OF AROMA-FLAVOR | SCORE OF BODY-TEXTURE | ACIDITY | VISCOSITY TIME |
	Cooling medium	Temperature after cooled				
		°F.	per cent	per cent	per cent	seconds
I	Tap water	72	79 00	77.80	0.897	113
II	Ice water	72	79.30	77 80	0.903	120
III	Surface cooler	72	77.60	78 00	0.901	109
IV	Surface cooler	50	78.00	77.70	0 901	113

nificant and show little relationship to the treatment of the different lots.

Wheying off did not occur in any of the lots other than a very slight amount formed on the surface, and was not considered harmful to the quality of the fermented milk. Cooling by different processes had no effect on the appearance of the samples.

Storage of the samples for a period of five days produced no whey and did not effect the appearance of the samples.

The effect of the pasteurization exposure on the properties of fermented reconstructed milk

Six series, of 4 lots each, were made to determine the effect of the pasteurization exposure on the fermented milk.

Table 4 shows the effect of the pasteurization exposure at the time of breaking the curd. Increasing the pasteurization temperature above 180°F., or exposing the milk for a period exceeding thirty minutes decreased the score of the aroma and flavor by causing a powder taste and odor to become apparent. The cooked aroma and flavor so often appearing in normal milks when heated to a high temperature did not appear in the reconstructed milk.

TABLE 4

The effect of the pasteurization exposure on the properties of fermented reconstructed milk

| NUMBER OF LOT | PASTEURIZATION | | SCORE OF AROMA-FLAVOR | SCORE OF BODY-TEXTURE | ACIDITY | VISCOSITY TIME |
	Temperature	Exposure				
	°F.	minutes	per cent	per cent	per cent	seconds
I	180	30	79.60	84 20	0.859	91
II	180	60	79 00	84.60	0 863	96
III	200	30	77.60	84 20	0 863	103
IV	200	60	74 00	83 80	0 872	127

TABLE 5

The effect of the addition of gelatine on the properties of fermented reconstructed milk

NUMBER OF LOT	GELATINE	SCORE OF AROMA-FLAVOR	SCORE OF BODY-TEXTURE	ACIDITY	VISCOSITY TIME
	per cent	per cent	per cent	per cent	seconds
I	0 00	80.10	86 60	0 890	109
II	0.15	80.80	88.30	0 890	126
III	0 25	80 00	89 60	0 896	149
IV	0 35	81 60	92 50	0 904	206

The score of the body and texture was not materially affected by the different pasteurization temperatures or periods of exposure. A slight increase in acid and a considerable increase in the viscosity resulted with an increase in the time or temperature of the pasteurization exposure.

Increasing the pasteurization exposure imparted a creamy appearance to the fermented milk. Whey formation was very limited and equally distributed among the samples.

There was no apparent relationship between the length of the pasteurization period, and the amount of whey formed or the appearance of the samples.

The effect of the addition of gelatin on the properties of the fermented reconstructed milk

Six series, of 4 lots each, were made to determine the effect of the addition of small amounts of gelatin on the properties of fermented reconstructed milk.

Table 5 shows the improvement of the properties of the fermented milk at the time of breaking the curd. The score of the aroma and flavor was not materially affected by the addition of gelatin. The presence of gelatin in small amounts caused a marked increase in the body and texture score of the samples. With each additional increment of gelatin there was a slight increase in the acidity of the samples. A marked increase in the viscosity is shown with increasing amounts of gelatin. Lot IV was increased almost one hundred per cent in viscosity time over lot I.

Lots III and IV appeared much firmer in curd than lots I and II; Lot IV in two instances was described as having a somewhat jelly-like consistency.

No appreciable amount of whey formed in any of the samples.

The effect of the addition of normal skim milk on the properties of fermented reconstructed milk

Four series, of 4 lots each, were made to determine the effect of the addition of normal skim milk on the properties of the fermented reconstructed milk.

The addition of normal skim milk to reconstructed milk increased the desirability of the fermented milk at the time of breaking the curd, as shown by table 6. The scores of aroma, flavor, body, and texture were constantly higher in those lots to which normal skim milk had been added before fermentation. Normal skim milk seemed to impart a richer aroma and flavor, and a more velvety body and texture to the fermented milk.

The acidity and the viscosity time of the fermented milk were not materially effected by the addition of different volumes of normal skim milk.

Wheying off did not occur in any of the lots and the appearance of all lots was uniform.

The keeping qualities of the fermented milks were much the same. The lots to which normal skim milk had been added, maintained their initial advantage in the scores of aroma, flavor,

TABLE 6

The effect of the addition of normal skim milk on the properties of fermented reconstructed milk

NUMBER OF LOT	AMOUNT OF NORMAL SKIN	SCORE OF AROMA-FLAVOR	SCORE OF BODY-TEXTURE	ACIDITY	VISCOSITY TIME
	per cent	*per cent*	*per cent*	*per cent*	*seconds*
I	None	81 20	89 50	0 890	152
II	10	83.20	91 20	0 892	151
III	20	87.50	94.50	0.893	155
IV	30	88.50	94 50	0 891	153

TABLE 7

The effect of cooling before breaking the curd on the properties of fermented reconstructed milk

NUMBER OF LOT	TIME BEFORE BREAKING CURD	SCORE OF AROMA-FLAVOR	SCORE OF BODY-TEXTURE	ACIDITY	VISCOSITY TIME
	hours	*per cent*	*per cent*	*per cent*	*seconds*
I	0	79 00	82.50	0 867	93
II	4	80.00	83.50	0 862	· 89
III	6	80.70	84 70	0.870	93
IV	8	81.00	87 00	0.878	91

body, and texture; all lots depreciating in score for twenty-four hours rather rapidly, with a subsequent slower rate of decline in value for the period of five days.

Storing the samples produced no whey and the appearance of the samples of fermented milk remained much the same.

The effect of cooling before breaking the curd on the properties of fermented reconstructed milk

Four series, of 4 lots each, were made to determine the effect of cooling before breaking the curd. All lots were then sampled and tested.

The effect of cooling after incubation, on the properties of the fermented milk is shown in table 7. A slight increase in the scores of aroma, flavor, body, and texture was recorded in those lots stored for longer periods before breaking the curd. The gain in score in every instance increased with the time stored before breaking the curd.

The acidity of the samples held for longer periods of time before breaking the curd increased slightly. The viscosity time, however, was not effected by cooling before breaking the curd.

TABLE 8

The keeping qualities of fermented milk stored at 39°F.

NUMBER OF LOT	STORAGE	SCORE OF AROMA AND FLAVOR	SCORE OF BODY AND TEXTURE	ACIDITY	VISCOSITY TIME
	hours	*per cent*	*per cent*	*per cent*	*seconds*
I	0	84.00	88.00	0 872	158
II	0	83.50	84.50	0.882	118
III	0	83.00	80.50	0.875	104
I	24	86.00	90.00	0.882	208
II	24	86.00	86.00	0.905	142
III	24	85 50	84.20	0.873	106
I	72	88.50	90.00	0.862	268
II	72	88.50	86.00	0.875	216
III	72	87.50	85.00	0 890	114
I	120	85.50	88.50	0.852	248
II	120	87.00	87.50	0.862	157
III	120	84.50	84.50	0.875	140

Only a very slight amount of whey formed in any case, and that formed in the individual samples, showed no relation to the treatment of the curd.

Storage of the samples produced no evidence of whey formation and no change in the appearance of the samples.

A comparison of the properties of fermented milks made from gelatinated reconstructed skim milk, normal skim milk, an reconstructed skim milk at various storage temperatures

The reconstructed skim milks were made by the outlined procedure. The gelatin was added to the skim milk powder

before the addition of water. All milks were made into fermented milk by the same process.

Two series, of 3 lots each, were made to furnish data for comparative purposes. The data given are the averages of the two series.

Lot I was made from reconstructed skim milk to which 0.35 per cent of gelatin, of 175 Bloom test, was added.

Lot II was made from normal skim milk.

Lot III was made from reconstructed skim milk, to which no gelatin was added.

TABLE 9

The keeping qualities of fermented milk stored at 60°F.

NUMBER OF LOT	STORAGE	SCORE OF AROMA AND FLAVOR	SCORE OF BODY AND TEXTURE	ACIDITY	VISCOSITY TIME
	hours	*per cent*	*per cent*	*per cent*	*seconds*
I	0	84 00	88.00	0 872	158
II	0	83 50	84.50	0 882	118
III	0	83 00	80.00	0.875	104
I	24	82 50	85 50	0.907	119
II	24	81.50	82.50	0 930	90
III	24	81 00	81.00	0.912	65
I	72	81.00	83 00	0 925	134
II	72	79 50	77 00	0.935	90
III	72	78.50	75.50	0 910	67
I	120	78.50	83.50	0.922	84
II	120	79.50	75.00	0.932	
III	120	77.00	73.50	0.902	

At the time of breaking the curd 12 samples were taken from each lot. Three samples were stored at 37° to 39°F., 3 at 58° to 60°F., 3 at 72°F., and 3 were stored at room temperature.

Tables 8 to 11, inclusive, show the properties of the fermented milks at the time of breaking the curd and during the storage period of five days.

Storing the samples at 39°F., as shown by table 8, resulted in an increase in the scores of the aroma, flavor, body, and texture during the seventy-two-hour period, followed by a decline in the

scores at the end of the five-day period.   Lots I and II showed
the same general trend in the viscosity time, while Lot III con-
tinued to gain through the five-day period.

TABLE 10

The keeping qualities of fermented milk stored at 72°F.

NUMBER OF LOT	STORAGE	SCORE OF AROMA AND FLAVOR	SCORE OF BODY AND TEXTURE	ACIDITY	VISCOSITY TIME
	hours	per cent	per cent	per cent	seconds
I	0	84.00	88.00	0.872	158
II	0	83.50	84.50	0.882	118
III	0	83.00	80.50	0.875	104
I	24	80.00	82.00	0.907	54
II	24	80.00	80.00	0.932	41
III	24	78.50	79.00	0.912	35
I	72	76.50	78.50	0.925	31
II	72	76.50	75.50	0.957	25
III	72	74.00	75.00	0 942	26

TABLE 11

The keeping qualities of fermented milk stored at room temperature

NUMBER OF LOT	STORAGE	SCORE OF AROMA AND FLAVOR	SCORE OF BODY AND TEXTURE	ACIDITY	VISCOSITY TIME
	hours	per cent	per cent	per cent	seconds
I	0	84.00	88.00	0.872	158
II	0	83.50	84 50	0.882	118
III	0	83 00	80.50	0.875	104
I	24	82 50	83.00	0.915	80
II	24	82.00	81.00	0.930	63
III	24	79.50	78.50	0.905	46
I	72	79.00	81.50	0.915	55
II	72	79.00	78.50	0.950	38
III	72	74.00	75.00	0.917	33
I	120	75.50	80.00	0.905	57
II	120	75.50	75.50	0.937	32
III	120	72.50	75.00	0.900	25

The acidity of lots I and II developed somewhat during the
first twenty-four hours of storage, followed by a decline during

the remainder of the storage period. Lots III continued to gain in acidity for three days, followed by a decline at the end of the fifth day.

Storage of the samples at 60°F., as shown by table 9, resulted in a gradual decline in the scores of the aroma, flavor, body, and texture during the storage period of five days. The acidity increased gradually during the seventy-two-hour period, followed by a decrease at the end of the fifth day. The viscosity of all lots showed a marked decrease at the end of the first 24 hours storage, followed by a tendency to remain constant in the instances of lots II and III, while lot I experienced some fluctuation.

Storage of the samples at 72°F. as shown by table 10, resulted in a very rapid decline in the properties of the fermented reconstructed milk. The score, of aroma, flavor, body, and texture show marked decreases at the end of the twenty-four hours storage, and further decreases at the end of the three-day period.

Storage of the samples at room temperature resulted in a general decline in the aroma, flavor, body, texture, and viscosity time, as shown by table 11; the acidity increasing for three days followed by a decrease at the end of the fifth day. The changes taking place in the samples held at room temperature were much the same as those taking place in the samples held at 60°F.; however, the changes proceeded at a faster rate, particularly during the seventy-two-hour and the one-hundred-and-twenty-hour period.

DISCUSSION OF EXPERIMENTAL DATA

Increasing the amount of inoculum improved the aroma and flavor and increased the acidity of the fermented milk made from reconstructed skim milk. Cordes and Hammer (12) found, in the fermentation of normal skim milk, higher total acidities resulted in the production of a higher percentage of volatile acids by S. Lacticus and the organisms associated with it in commercial cultures. The same is no doubt true of fermented reconstructed skim milks. The increased amount of volatile acid present may account for the improvement in aroma and flavor in the fermented milks made from reconstructed skim milk inoculated with higher percentages of starter.

An increase in the amount of inoculum resulted in the improvement in the body and texture of the fermented milk and in a slower rate of deterioration of the body on storage, due to a more complete fermentation. The weakness of the curd due to low acid development would reflect itself in the score of the body and texture, and caused a more rapid deterioration in the body and texture of the fermented milk on storage.

The method used and the rapidity of reducing the temperature of the milk after pasteurization had little effect on the properties of the fermented milk except that the surface cooler method which has an advantage in point of time to cool, and in the aeration of the pasteurized milk, endangers the milk by possible contamination.

Increasing the pasteurization exposure of the milk to be fermented results in an inferior aroma and flavor in the fermented milk. The slight powder taste and odor common to all desiccated milks and present at all times in the fermented milk, is intensified by high temperatures or even moderate temperatures (180°F.), on long exposure, and becomes more apparent in the fermented milk on storage, probably due to the loss of volatile acids.

The addition of small amounts of gelatin to the reconstructed skim milk to be fermented results in an improvement in the body and texture of the fermented milk. The presence of a gel incorporating the curd produces a heavier, more viscous body, which would retard the formation of whey, and cause a slower rate of deterioration of the body.

That the nature of the proteins of milk are changed on dessication was shown by Dahle and Palmer (3). The change in the nature of the proteins reflects itself in the formation of a weaker curd on fermentation as shown by table 6. The addition of normal skim milk in relatively large quantities would cause a more normal fermentation to take place and would account for the formation of a firmer curd with a consequent increase in the score of the body and texture and an improvement of the aroma and flavor.

Cooling the fermented milk before breaking the curd improves the aroma, flavor, body, and texture of the product as shown by

table 7. The added fermentation taking place, as shown by the increased acidity present, would be a large factor in the improvement in the fermented milk due to the presence of more volatile acids, and to the formation of a firmer curd.

A comparison of the properties of fermented milks made from gelatinated reconstructed skim milk, normal skim milk, and reconstructed skim milk to which no gelatin had been added, table 8, shows them to be nearly equal in the scores of aroma and flavor. The apparent ability of *S. Lacticus* to grow better in normal skim milk with the production of more volatile acids probably accounts for the richer aroma and flavor of the fermented milk made from normal skim milk.

The body and texture of the lots made from gelatinated reconstructed skim milk were judged to be superior to the lots made from normal skim milk. The presence of a slight lumpiness decreased the score of the body and texture in those lots made from normal skim milk. The lumpiness of the lots made from the normal skim milk was not considered as objectionable as the weakness of the lots made from reconstructed skim milk to which no gelatin had been added.

Storage of fermented milks at temperatures of 60°F. or above, will result in the rapid deterioration of the product as those temperatures are suitable for the growth and development of different types of bacteria. The development of *S. Lacticus* and associated groups is inhibited by a high acidity development as pointed out by Hammer (6). However, the growth of other organisms that may have escaped the pasteurization exposure is not inhibited and may result in the production of off aromas and flavors and in the disintegration of the body by proteolytic bacteria.

CONCLUSIONS

1. A fermented milk possessing very desirable qualities can be made from reconstructed skim milk when the processes pertinent to its manufacture are recognized and uniformly controlled.

2. Fermented milks made from reconstructed skim milk are characterized by a body and texture that are consistently very smooth.

3. The quality of the fermented milk may be improved or impaired by modifying the manufacturing process.

4. The quality of the starter used is a factor of utmost importance. An amount of high quality starter as practical in commercial practice should be used.

5. The cooling process subsequent to pasteurization has no important effect on the quality of fermented milk made from reconstructed skim milk.

6. Pasteurization of the reconstructed skim milk at high temperatures or for long exposures should be avoided.

7. The addition of 0.35 per cent of gelatin can be recommended as improving the body and texture of the fermented milk made from reconstructed skim milk.

8. The addition of normal skim milk in amounts exceeding 10 per cent can be recommended.

9. Cooling of the fermented milk prior to the breaking of the curd should be practiced whenever possible.

10. Storage of fermented milk made from reconstructed skim milk at a temperature of 39°F. causes no deterioration in the quality of the product in five days.

11. Storage temperatures above 42°F. causes a decrease in quality after three days while a storage temperature of 60°F. results in an inferior product in twenty-four hours.

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α AND β LACTOSE IN SOME MILK PRODUCTS[*]

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INTRODUCTION

The α hydrate form of lactose has been known for a long time. It is this form which crystallizes from sweetened condensed milk and ice cream, and in the commercial manufacture of milk sugar.

Erdmann (4) in 1855 was the first to call attention to another form of lactose which we now recognize as the β form. It crystallizes from a concentrated solution of lactose at the boiling point. He observed that the optical rotation of a freshly prepared solution of β lactose increased with time while a freshly prepared solution of α lactose decreased with time. Dubrunfant (3) also observed the decrease in rotation of freshly prepared α lactose solutions. Interest in the β form of lactose was revived some time later by Schmoger (21) (22) and Erdmann (5). Erdmann's method of preparing β lactose by evaporating a solution to dryness resulted in a browned impure product. Tanret (25) tried to improve upon Erdmann's method by redissolving the β lactose in water and reprecipitating with alcohol. Tanret and others thought there were three forms of lactose. Hudson (11) (12) studied the solubility of lactose under varying conditions and showed that the third form was a mixture of α and β lactose. Gillis (6) gives phase diagrams for lactose-water systems and also additional information on the solubility of lactose.

Lactose tends to form supersaturated solutions. Attention was called to this behavior as early as 1856 by Debrunfant (3). The supersaturation which can ordinarily be obtained has been shown by Leighton and Peter (17). The effect of sucrose on the solubility of lactose has been studied by Hunziker and Nissen (14)

and Peter (19).   An extensive review of the general literature on lactose has been given by Whittier (30).

A study of lactose has been in progress for some time in this laboratory. Sharp (23) attempted to point out the possible significance of the rate of change of β to α lactose as an important factor in controlling the crystallization of lactose from condensed milk, ice cream and concentrated lactose solutions. This paper is a preliminary report of a phase of this work dealing with the relative amounts of α and β lactose in dairy products, together with a study of some factors causing a change in the relative amounts of the two forms.

EXPERIMENTAL

The effect of the pH of the solution on the rate of conversion of the forms of lactose into each other

The optical rotation of a freshly prepared solution of α lactose is higher than it is after standing for some time, the decrease in rotation being due to the change of some of the α into the β form. The specific rotation of the β form is much lower than is the specific rotation of the α form.   The specific rotation of α lactose is 86.0° and of β lactose 35.4° at 20°C. according to Hudson (12). Erdmann (4) and Urech (27) observed that acids accelerated the rate of decrease in rotation of α lactose solutions and Urech observed that ammonium hydroxide caused the change to be almost instantaneous. Trey (26) investigated the effect of a number of substances as influencing the rate of change of rotation of lactose solutions.   He clearly showed that alkalies accelerate the rate of change more than do acids and if too much alkali is added decomposition begins.   Bleyer and Schmidt (1) stated that very strong acid solutions displaced the equilibrium toward the formation of α lactose while strong alkalies displaced it toward the formation of β lactose.

It seems that these various substances for the most part exert their accelerating effect on the rate of change of the forms of lactose into each other by a change in the hydrogen ion concentration, therefore, the influence of pH upon this behavior was determined.

The change of the two forms of lactose into each other can be followed quite conveniently by means of the polariscope. Urech (28) called attention to the monomolecular character of this reaction. It is an incomplete reaction which comes to equilibrium when there is present in the solution about 1.58 parts of β to 1.0 part of α lactose at room temperature. The rate of change is given by the following equation:

$$\frac{dx}{dt} = k_1 (a - x) - k_2 (b + x)$$

Where a is the concentration of the α form at the beginning, b the concentration of the β form at the beginning and x is the amount of α form which has changed to the β form in the time t, k_1 is the reaction velocity constant for the change of α to β and k_2 is the reaction velocity constant for the change of β to α. The above equation after integration and simplification can be made to take the following form in which the reaction is followed by means of the polariscope.

$$\frac{1}{t} \log \frac{R_0 - R}{R_t - R} = k_1 + k_2$$

where R_0 is the rotation at the start R is the rotation after equilibrium has been reached and R_t is the rotation at the time t expressed in hours. For the purpose of comparison K was used in place of $k_1 + k_2$ and common logarithms were used in place of the natural logarithms. This equation is presented in the form used by Hudson (11). Hudson's (10) temperature equation gives 0.475 for the value of K at 25°C. Recalculating some of Trey's (26) data the value for K obtained was 0.478. Verschuur's (29) data gives 0.472 when the rate was determined using α lactose and 0.474 when β lactose was used. We have obtained values in the neighborhood of 0.473 at pH 6.0, at pH 4.5 the value is more nearly 0.460. By dissolving lactose in distilled water some variations in the constant were observed perhaps due to fluctuations in pH of such unbuffered solutions.

In most of our experimental work α lactose was used as the starting material. All rotations were observed at 25°C. using
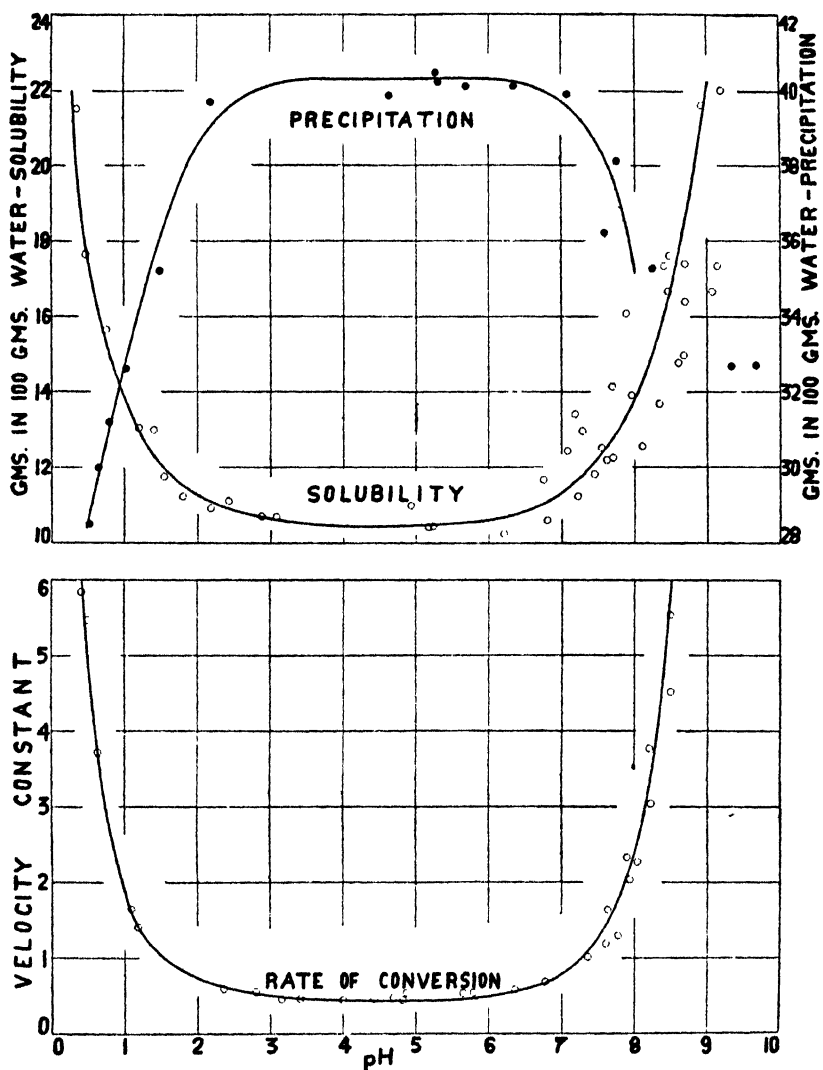
FIG. 1. THE EFFECT OF pH ON THE RATE OF CHANGE OF THE FORMS OF LACTOSE
INTO EACH OTHER AS INFLUENCING THE RATE OF SOLUTION AND
PRECIPITATION OF LACTOSE

a jacketed 2 decimeter tube. The different pH values were obtained with dilute buffer solutions except in the high acid range where hydrochloric acid alone was used. The results obtained are expressed graphically in figure 1.

This figure shows that within the range which is normally encountered in dairy products, the pH of the solution would exert little effect upon the rate of change of lactose from one form to the other. The curve is fairly symmetrical with a rather wide middle region displaced to the acid side of neutrality, in which a change in pH produces little effect upon the rate of change of the one form of lactose into the other. The rate of change approaches infinity on the acid side at pH 0.0 and on the alkaline side at pH 9.0.

Mills and Hogarth (18) found that milk sugar (α lactose hydrate) has two solubilities which they term "initial" and "permanent" solubility. They show that the initial solubility is obtained at once and is considerably less than the permanent solubility which was obtained only after a long time.

This curve for the effect of the pH of the solution on the rate of change of the two forms of lactose into each other shows that, in the pH region represented by the horizontal portion of the curve, the attainment of the "permanent" solubility of lactose would be slow while at the two ends of the curve where the rate increases sharply the attainment of the permanent solubility of lactose would be rapid. Urech (28) recognized the importance of this change of α lactose hydrate into another form as influencing the rate of solution of lactose. He found that lactose reached its permanent solubility rapidly in acids and alkalies because they accelerated this rate of change. In our experiments this effect was easily demonstrated by shaking solutions of various pH values with an excess of α lactose hydrate at 25°C. for ten minutes, filtering and determining the concentration of lactose in the filtrate. The results obtained in this way are plotted as the "solubility" curve in the upper part of figure 1. It will be observed that this curve is very similar to the rate of change curve in the lower part of figure 1. A more concentrated solution is obtained in a shorter time at the extremes of the curve because solutions of

the corresponding pH values accelerate the rate of change of α into β lactose:

Lactose should crystallize most rapidly from supersaturated solutions in the pH regions where the rate of change of the forms of lactose into each other is most rapid. In two experiments Jenkins (15) showed that the velocity of crystallization of α lactose hydrate was faster in ammonium hydroxide solutions than it was in water. The effect of pH in our experiments was demonstrated by taking 50 cc. of a solution of lactose saturated at 50°C., cooling it down to 25°C., adjusting its pH and adding 5 grams of α lactose hydrate as seeding material. The mixture was then agitated at 25°C. for thirty minutes, at the end of this time the solution was filtered and the concentration of the solution determined. The results obtained are plotted as the "precipitation" curve in the upper part of figure 1. It will be observed that the concentration of the solution decreased most in the regions where the change of the forms of lactose into each other was most rapid. Precipitation in the region of pH 9 is not as rapid as would be expected, indicating the effect of another factor, possibly due to a reaction between the lactose and the alkali. This precipitation curve is the reverse of the solubility curve. A considerable lag in the precipitation of α lactose hydrate was observed even though the solution was thoroughly seeded and agitated continually. There was much less lag in the solubility curve.

The data in figure 1 show that within the range which is normally encountered in dairy products changes in pH will exert little effect on the solution and precipitation of α lactose hydrate in so far as the pH effects the rate of change of the forms of lactose into each other.

The effect of concentrated sucrose solutions on the rate of conversion of the forms of lactose into each other

Ice cream mixtures and condensed milk contain considerable amounts of sucrose. Hunziker and Nissen (14) found that concentrated sucrose solutions decreased the solubility of lactose slightly at 18°C. while Peter (19) found that the solubility of

lactose in a concentrated sucrose solution was appreciably decreased at near the freezing point.

It was conceivable at least that high concentrations of sucrose might affect the rate of change of the forms of lactose into each other. The effect, if any, is however very small. Trey (26) gives data which yield the value for K of 0.478 for the velocity constant in water at 25°C. In his experiment in which 4.5 grams of α lactose and 17.1 grams of sucrose were made up to 100 cc. with water, the value for K was 0.435 and when β lactose was used the value obtained was 0.462. These values are probably all within the limit of error of Trey's experiments.

The average velocity constant of 0.46 was obtained in a number of experiments in which α lactose hydrate was dissolved in water at a pH in the neighborhood of 5.0. When α lactose hydrate was dissolved in about 40 per cent sucrose solutions at the same pH, the values for K obtained in different experiments were 0.454, 0.456, 0.461, 0.458, and 0.461.

It appears that at 25°C. at least, the presence of considerable amounts of sucrose in the solution has little or no effect on the rate of conversion of the two forms of lactose into each other.

Lactose in dried milk

Dried skimmilk is approximately half lactose and consequently one might expect that some of the properties of lactose would be reflected in the properties of the dried milk. Hauser (7) and Hauser and Hering (8) studied the heat of wetting of dried milk of various moisture contents. They found that a break in the curve occured at about 3 per cent moisture content with fresh whole milk powder. They interpreted this break as indicating the place at which the lactose became all hydrated. Different curves were obtained with different types of powders. They recommended the determination of the heat of wetting as a quick method for the estimation of the moisture content of milk powder.

Holm and Greenbank (9) and Supplee (24) have determined the moisture content of dried milk obtained at a series of constant humidities. Holm attributed a difference in moisture content which he found when atmospheric roll and spray dried powders

were held at a constant humidity to a difference in heat treatment of the protein material during the drying process. Supplee discovered a very interesting behavior, namely, that the hydration and dehydration moisture contents of the same powder at the same humidity were quite different. This led him to hold samples of dried milk at humidities in the neighborhood of 50 per cent and he found that samples held at constant humidity at first took up moisture and then gave up a considerable portion of it again.

A study was begun in this laboratory to see if some of these peculiar behaviors of dried milk might not be due to lactose instead of the proteins as previous investigators were inclined to believe.

Little information is available as to the state of lactose in dried milk. Hauser and Hering (8) say that it is amorphous in the fresh Krause product but indicate that at 3 per cent moisture content it is present as the hydrate. They also mention one powder in which part of the lactose was present as the hydrate and another in which all was present as the hydrate as determined by the polarizing microscope.

We found that by dissolving dried milk in water and then quickly precipitating the proteins with a solution of mercuric chloride and alcohol a clear filtrate containing the lactose in solution was obtained. The filtrate was immediately placed in a polariscope tube and the change in rotation was determined. From these readings the amount of lactose present in the α and β forms was calculated. These readings were all made at 25°C. using a water jacketed polariscope tube.

It was found that the fresh dried skimmilk prepared by the pressure and centrifugal spray and the atmospheric and vacuum drum processes contained approximately the same proportions of α and β lactose as does ordinary fluid skimmilk. The rotation usually fell slightly but this was probably due to the fact that the milk is not dried at 25°C. but at some higher temperature where there is a slightly greater proportion of α lactose as compared with β lactose. This would be expected from the work of Gillis (6) who has shown that the ratio of β/α lactose at equilibrium

varies from 1.65 at 0°C. to about 1.33 at 100°C.; at 25°C. the ratio is about 1.58.

The results obtained by polarizing the solutions prepared from the fresh skimmilk powders listed above indicate that the milk dries before the lactose starts to crystallize or at least dries before any crystillization and change of one form into the other can take place. That the lactose in fresh skimmilk dried by the above mentioned processes is not crystalline was shown by a seeding test which will be described later and by observation with the polarizing microscope.

Skimmilk dried by the flake process when dissolved and subjected to the polariscopic examination described above showed a considerable fall in rotation, indicating that the α hydrate form of lactose had actually crystallized out in this process of drying and that an appreciable amount of β lactose had changed to α lactose before the drying proceeded far enough to stop further change. The presence of α lactose hydrate crystals was confirmed by the seeding test. While possibly not all of the lactose in the α form in the flake process powder is in the crystalline condition yet an amount of α lactose which corresponds to the lactose which has changed from the β to the α form must have necessarily crystallized out. It is probable that most of the α lactose is in the crystalline form in this type of dried milk. The crystallization of lactose in the flake dried skimmilk is started by the continuous seeding of the milk as it enters the drier, due to the mechanics of the way in which the drying is carried out.

Supplee (24) found that the humidity-dehydration curve lies above the humidity-hydration curve when the dried milk was hydrated at 80 per cent humidity.

Our study shows that this result was due in part, at least, to the presence of α lactose hydrate crystals with their 5 per cent of water which would not be removed at any of the humidities used in his dehydration studies.

It was found that concentrations above about 85 per cent sulphuric acid will dehydrate α lactose hydrate crystals at room temperature if given sufficient time. A concentration of about 85 per cent sulfuric acid does not dehydrate α lactose hydrate,

crystals, and yet it removes a considerable amount of moisture from ordinary dried milk. Samples of 4 kinds of dried milk have geen maintained over 85 per cent sulfuric acid for two years and they are still changing in weight. At the present time the moisture contents are: flake 1.97 per cent, pressure spray 1.17 per cent, atmospheric drum 0.96 per cent, and vacuum drum 1.01 per cent. The flake powder has the highest moisture content because of the presence of a considerable amount of α lactose hydrate crystals, the water from which is not removed by 85 per cent sulfuric acid. The range of variation in moisture content of the other 3 kinds of dried milk is 0.21 per cent. This experiment was repeated with an entirely different set of samples with the same result.

Some of the behaviors of dried milk reported by Hauser and Hering (8) can be partially accounted for, at least, on the basis of the crystallization of the lactose. For example, the difference in behavior of dried milk held only a short time and a longer time at the high humidities, and the behavior of dried milk in which the moisture has been increased to a considerable extent and then decreased again, also the differences between the dried milk samples which they cite as containing different amounts of crystalline hydrate.

The lactose in pressure and centrifugally spray dried whey is also present in a non-crystalline equilibrium mixture of α and β lactose.

Theoretically, at least, an indication of the temperature at which the powders are dried can be gained from the ratio of β to α lactose present in the non-crystalline dried material.

Evidence that the lactose in the freshly dried milk by the spray and drum processes is present in the form of a very concentrated syrup seems to be abundant and conclusive. Solution and polarization indicated that the two forms of lactose are in equilibrium. In attempting to dry whey by the roller process a "glass" is obtained on the rolls. Very good evidence that the lactose is not crystalline comes from the humidity equilibrium experiments which will be explained later. The absence of α lactose hydrate crystals in spray and roller dried milk and in spray dried whey is shown by a seeding test, and by the polarizing microscope.

Lactose forms fairly stable supersaturated solutions in the absence of seeding nuclei. A solution of lactose saturated at 50°C. was filtered and cooled to 20°C. Ten cubic-centimeter portions of this solution were placed in test tubes and small amounts of the dried milks (about 25 mgm.) being tested were added to each tube. The tubes were stoppered and shaken at intervals for about one hour. During this time the solution of lactose will begin to crystallize if seeding crystals of α lactose hydrate are present in the material added. The fresh spray and drum dried skimmilk and the spray dried whey show no crystallization of the solution when this test is applied but flake dried skimmilk induces copious crystallization. Spray and drum dried milk powders of the various kinds as well as spray dried whey show copious crystallization when this test is applied provided they have once definitely caked. The seeding test was previously described by Hudson and Brown (12) for detecting the presence of α lactose hydrate in β lactose preparations.

Prof. C. W. Mason was kind enough to examine a series of dried milk and dried whey samples for crystallization, with the polarizing microscope. His observations as to the presence or absence of crystals were a complete confirmation of the results obtained by the seeding test with the exception that occasionally a crystal of some material was observed in samples which gave a negative seeding test at the end of one hour.

The caking of dried milk

Schmoeger (22) reported that if a pure lactose solution was dried very rapidly a non-crystalline "glass" was obtained which took up moisture very readily, became sticky and then crystallized to a hard mass of α hydrate lactose.

Supplee (24) has shown that in the neighborhood of 50 per cent relative humidity dried milk will first take up moisture and then give up some of it again. We have confirmed his observation and shown that dried whey also does the same thing. After these products have taken up moisture and then given it up again it is found that they have caked. Since the lactose in dried milk and whey is present in the form of a very concentrated syrup it

consequently produces a solution with a very low vapor pressure or very high osmotic pressure. This solution of lactose is so concentrated that the lactose cannot crystallize and if the moisture content of the milk powder is maintained at a low enough level crystallization will not occur and the milk powder does not cake. Since such a concentrated syrup is very hygroscopic it tends to take up water when exposed to atmospheres which con-

TABLE 1

Distribution of the two forms of lactose in dried milk after storage for five months at 70 per cent humidity

KIND OF DRIED MILK	PREVIOUS TREATMENT	LACTOSE		β WHICH HAS BEEN CHANGED TO α	SEEDING TEST	POLARIZING MICROSCOPE, CRYSTALS
		α	β			
		per cent	*per cent*	*per cent*		
Open roll	Fresh	(38 7)	(61 3)	0	—	—
	5 months at 70 per cent humidity	53 1	46 9	23.5	+	+
Pressure spray	Fresh	(38 7)	(61.3)	0	—	—
	5 months at 70 per cent humidity	51.1	48 9	20.2	+	+
Vacuum roll	Fresh	(38 7)	(61 3)	0	—	—
	5 months at 70 per cent humidity	50 0	50 0	18 4	+	+
Flake	Fresh	63 0	37 0	39.6	+	+
	5 months at 70 per cent humidity	77 8	22 2	63 8	+	+

tain appreciable moisture, this water which the dried milk takes up dilutes the lactose solution tending to cause the milk particles to stick together and if enough water is taken up in this way the lactose solution is diluted to the concentration where it can crystallize. After crystallization begins the concentration of the solution is decreased due to the solid phase separating out; this decreases the osmotic pressure and increases the vapor tension causing some of the moisture to be given up again. This lactose which crystallizes out is, of course, α hydrate and crystallizes with a molecule of water. The crystallization causes a caking of

the product.   In the case of dried whey in which this process can be followed with ease, the material may become plastic and when crystallization occurs the product becomes quite hard.

The change of β to α lactose does not reach completion except possibly after a very long time.   Samples have been under observation for two years and they are still changing.   This is perhaps due to slow diffusion through the nearly solid syrup.   Some idea of the extent of this change is given in table 1.   This table shows the change of β to α lactose in milk powders held for about five months at a humidity of approximately 70 per cent.   These powders first took up moisture and then gave up some of it again.

The fresh samples of open and vacuum roll and spray powders are assumed to be in equilibrium with a ratio of 1.58 parts of β to 1.0 part of α.   Actually the rotation of such fresh powders may indicate a slightly higher proportion of α lactose but the fall in rotation is only a few hundredth of a degree.   The seeding tests have been negative.

Crystallization of lactose in ice cream

Bothell (2) and Zoller and Williams (31) showed that sandy ice cream was caused by the crystallization of lactose hydrate. Sharp (23) presented an explanation for the crystallization and development of α lactose hydrate crystals in ice cream based on the assumption that both α lactose hydrate and β lactose anhydride crystallized out and that the growth of the α lactose hydrate crystals was controlled by the effect of temperature on the rate of change of β to α lactose.   Although this explanation seemed to agree with the general development of "sandy" ice cream, it was shown to be incorrect by the very simple seeding test.   Ice cream may be held for a very appreciable period and yet be free from crystals of α lactose hydrate which induce copious crystallization of a supersaturated lactose solution.   Sandy ice cream does not produce such clear cut seeding action on a supersaturated solution of lactose as do some other milk products.

The amounts of α and β lactose in ice cream can be determined by means of the polariscope using the same procedure as was described for dried milk.   It was found that in ice cream which

was not sandy, the ratio of β to α lactose was undisturbed and was approximately the same as in a solution of lactose at equilibrium at room temperature. The change obtained in the optical rotation was hardly detectable. In the case of sandy ice cream, however, there was always an excess of α, the excess increasing with the degree of sandiness. In one instance where a case of incipient sandiness was examined the excess of α was about six times the maximum allowable error in reading the polariscope, showing that sandiness can probably be detected and followed by means of the polariscope long before it can be detected organolyptically. Only two out of several experienced judges recognized the sandiness of this sample and then only after repeated trials.

The polariscopic method of following the development of sandiness in ice cream has distinct advantages. Any method of attempting to filter off the crystallized lactose from the freshly melted ice cream has the error that some of the lactose will go into solution before the separation can be accomplished. In using the polariscopic method, however, the lactose is all put back into solution and the excess of α determined from the polariscope readings.

The crystallization of α lactose hydrate in ice cream probably follows a course similar to the crystallization of α lactose hydrate in dried milk. The solubility limit of α lactose hydrate must be exceeded in order for it to crystallize in ice cream. The concentration of the lactose solution is accomplished by the freezing of water. A concentration of lactose in the unfrozen water of the ice cream exists where the α lactose hydrate is most susceptible to crystallization, if the solution in the ice cream becomes too concentrated crystallization cannot occur because not enough water is present to permit the lactose molecules to arrange themselves in a definite grouping which is necessary for crystal formation. Ordinarily the ice cream is drawn from the freezer and placed in a cold hardening room. The property of lactose by which it forms supersaturated solutions is an important factor. The freezing of water during the hardening process may concentrate the lactose solution until it cannot crystallize. Crystallization is made more difficult because of the mixture of three kinds

of sugar molecules in the syrup, namely sucrose, β lactose, and α lactose. If the ice cream is warmed to some extent ice melts and dilutes the syrup, this dilution may be to such an extent that the α lactose hydrate can crystallize. The conditions under which the crystallization first starts are probably influenced by seeding nuclei.

The forms of lactose in the dried residue in the total solids determination

Schmoeger (22) Hudson (12) Rice and Miscall (20) Hunziker and Nissen (14) and Koestler and Lörtscher (16) seem to be fairly well in agreement that the lactose in the dried residue in the total solids determination in milk is present as the anhydride. With the exception of Hudson (12) and Schmoeger, no clear indication is given as to which anhydride was meant. Schmoeger dried milk on sand, extracted the sand with a small amount of water, precipitated the proteins with lead acetate and polarized the filtrate. He found that the rotation decreased only slightly on standing. He concluded from this result that the lactose was present in the dried residue as a mixture of the two forms which we know as α and β lactose.

We have also found that the lactose is present in the dried residue from the total solids determination, as oridnarily made, as the equilibrium mixture of β and α lactose. Skimmilk was dried according to the method for determining total solids, (a) using the Mojonnier method, (b) by drying in a thin layer in a boiling water oven, and (c) by drying in a thin layer at 50°C. In all three cases the lactose in the residue was found to be the equilibrium mixture of α and β. When relatively large bulks of milk were preserved with mercuric chloride and dried slowly at 50°C. α lactose hydrate crystallized out, disturbing the equilibrium until eventually practically all of the lactose crystallized out as the α hydrate form.

These experiments indicate that the lactose in the residue in the total solids determination is present in a non-crystalline condition as a concentrated syrup which has become so concentrated that in ordinary analytical work it cannot be distinguished from the

anhydrides. If it were true that the α lactose hydrate crystallized out in the total solids determination of milk it would probably give a different value for the total solids depending upon the extent to which the water was removed from the crystalline α lactose hydrate.

SUMMARY AND CONCLUSION

1. Between the pH values of 2.0 and 7.0 the rate of change of α and β lactose into each other is at a minimum. The rate approaches infinity at pH 0.0 and 9.0.

2. The effect of pH on the rate of change of α to β lactose was shown to influence the rate of solution of α lactose hydrate and the effect of pH on the rate of change of β to α lactose was shown to influence the rate of precipitation of α lactose hydrate at 25°C.

3. Relatively concentrated sucrose solutions have little effect on the rate of change of the two forms of lactose into each other at 25°C.

4. Lactose was found to be in a non-crystalline equilibrium mixture of the α and β forms in skimmilk dried by the pressure and centrifugal spray and by the atmospheric and vacuum drum methods, when freshly prepared and maintained at a low moisture content. A considerable amount of α lactose hydrate was found to have crystallized in skimmilk during drying by the flake process.

5. The caking of milk powder is believed to proceed as follows: (1) the absorption of moisture by the concentrated lactose syrup, (2) the adherence of the milk particles to one another, and (3) the solidification of the mass due to the crystallization of some of the lactose as α hydrate.

6. The formation of "sandy" ice cream was explained as being due to the presence of sufficient non-frozen water to permit the α lactose hydrate to crystallize. By rapidly freezing out the water from ice cream, the lactose solution may be concentrated to a region where the crystallization of the α lactose hydrate does not occur. α lactose hydrate crystals form in such ice cream if sufficient ice is melted to dilute the syrup to a range of concentration where crystallization can take place.

7. The Hudson and Brown seeding test was shown to be useful

for recognizing the presence of crystalline α lactose hydrate in dairy products.

8. Lactose was found to be present in the dry residue of the total solids determination in milk as an equilibrium mixture of α and β lactose in the non-crystalline condition.

9. It was found that nearly all of the lactose crystallized out as the α hydrate form when milk was dried very slowly at 50°C.

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THE STERILIZATION OF SWEET CREAM FOR MARKET PURPOSES*

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The preparation of a sweet, sterile cream put into small containers, to be used for retail trade, offers certain technical difficulties. It is hoped that the results reported in this paper will help to overcome these and to facilitate the production of a cream which it is believed will have a definite and useful rôle in our increasing list of dairy products.

A method of preparing sterile cream has been described in a public patent issued to the author (1) and the fundamental relations governing the heat stability of homogenized creams have been investigated by Webb and Holm (2).

Sweet cream containing 20 per cent butterfat will withstand a sterilization temperature of 120°C. for 90 to 140 minutes before coagulation. To prevent separation of fat during long periods of storage however cream must be homogenized at relatively high pressures. Such treatment invariably lowers the heat stability of the product, due to factors as yet unexplained.

EXPERIMENTAL

To obtain a complete picture of the effect of homogenization upon the heat stability of cream containing 20 per cent butterfat, numerous samples were preheated to 60°, 70°, 80°, and 90°C. without holding, each being homogenized at pressures of from 500 to 4000 pounds as soon as the preheating temperature was attained. These samples were subsequently sterilized at 118°C. until coagulation occurred. Considerable variation in stability was found for different creams, but the relative effect upon stability of preheating before homogenization was the same. Representative data for a normal cream are plotted in figure 1.

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Variations in butterfat content were found to produce marked changes in stability after homogenization. Increases beyond 20 per cent lowered stability, whereas decreases increased it. Data showing this relationship for creams of three butterfat percentages are given in table 1. (See also Webb and Holm (2).)

Homogenization of cream containing 20 per cent butterfat at 2800 to 3200 pounds pressure will largely prevent separation of fat in storage. In the samples prepared a soft cream plug always
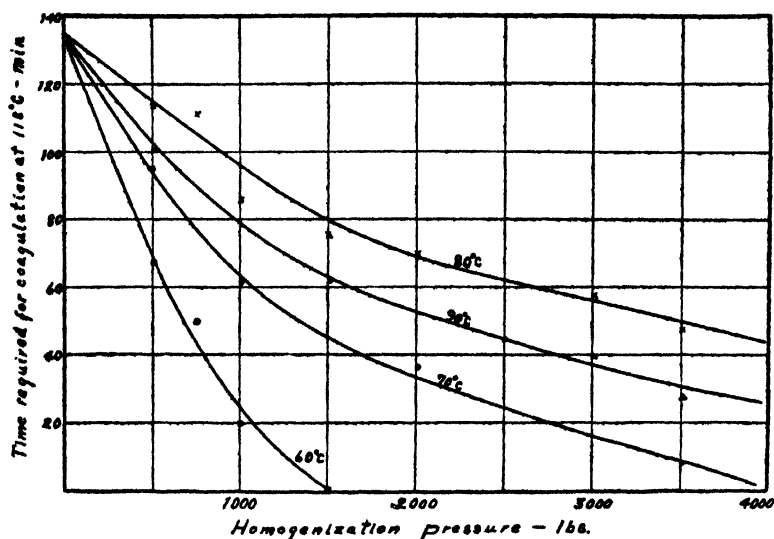

FIG. 1. EFFECT OF PREHEATING TEMPERATURE UPON THE HEAT STABILITY OF 20 PER CENT CREAM HOMOGENIZED AT VARIOUS PRESSURES

formed after a few months' undisturbed storage at room temperature. Nevertheless, this separation was not greatly increased even after two years, and the cream plug readily dissolved, especially when warm, with a little shaking. It was found that by storing the bottles upside down the cream plug more readily disintegrated upon shaking and in no way obstructed the narrow neck of the bottle.

Samples of sterile cream were scored for flavor, on the basis of 15 as perfect. This was also done for creams sterilized at differ-

ent times and temperatures and stored at room temperature for various periods up to 2½ years, when the flavor was still good and the cream would have passed as coffee cream or cream for cereal or fruit. Some of the representative data upon flavor are given

TABLE 1

Effect of variation in butterfat content upon the heat stability of creams preheated at different temperatures

PREHEATING TEMPERATURE	HOMOGENIZATION PRESSURE	TIME OF COAGULATION AT 118°C.		
		15 per cent butterfat	20 per cent butterfat	25 per cent butterfat
°C.	pounds per square inch	minutes	minutes	minutes
Not heated	0	140	136	128
60	3,000	27	1	−0
80	3,000	85	52	18

TABLE 2

Effect of time and temperature of sterilization and of time of storage upon the flavor of sterilized cream *

TEMPERATURE OF STERILIZATION	CHECK, FRESH CREAM, NO STERILIZATION	TIME OF STORAGE AT ROOM TEMPERATURE									
		24 hours			1 month			3 months			2½ years
		Time of sterilization in minutes									
		8	12	16	8	12	16	8	12	16	12
°C.											
108	13.5		11.9			10.1			9.5		6.8	Pronounced old cream flavor
112	13.5	11.7	11.1	10.7	10.0	10.1	10.3	9.9	9.9		0.0	Rancid
115	13.5	11.3	10.8	10.2	10.5	10.5	10.3	10.1	10.3	10.3	8.8	Old cream flavor
118	13.5	10.8	10.6	10.4	10.5	10.5	10.5	10.1	10.3	10.4	9.2	Old cream flavor
122	13.5		10.0			10.1			10.0		9.2	Old cream flavor

* Score for flavor on basis of 15 for perfect.

in table 2. Sterilization at 118°C. for 12 to 14 minutes seemed to produce a cream with the best keeping quality and flavor.

Several types of containers for the sterile products were used. It was found that when put up in tin cans, the cream always developed an undesirable rancid-like flavor, although when pieces of

tin were placed in the glass containers the cream did not have the flavor characteristic of that in the tin containers. The use of evacuated glass jars and of evacuated tin cans proved to have no more advantage in improving the flavor of the product after storage than did the same containers sealed in air.

The container finally decided upon as the most practical and as one which did not change the flavor of the cream was a 250-cc. white-glass bottle similar to a grape-juice or soda-water bottle. The caps used were lined with parchment or special cardboard rather than composition cork, which blackened the top layer of cream with which it came in contact. These bottles were easily sealed and withstood sterilization without breakage or loss of tops.

Observations upon the color of the product were made by comparison with fixed color tubes prepared according to Webb and Holm (3) by adding varying amounts of a 10 per cent $FeCl_3$ solution to a $1:5$ $CaCO_3$ suspension, to match cream colors.

There was a slight but noticeable darkening in color after sterilization and a very slight darkening up to the first month of storage, after which no significant change in color was detected.

Relative viscosity during storage was measured, but no important relationship was found between length of storage of the creams sterilized at various times and temperatures and viscosity.

An investigation of the whipping properties of sterile cream was carried out. The work of Babcock (4) on the whipping quality of cream was found of value in its application to sterile cream. Such creams were prepared with a butterfat content of from 20 to 35 per cent. When creams with a butterfat percentage above 30 were homogenized at more than 2000 pounds pressure they seldom withstood sterilization at 118°C. for 12 minutes. Heat treatment alone only slightly lowered whipping quality, but homogenization of the product seriously decreased the whipping ability of a cream, approximately in proportion to the pressure applied. Whipping temperature was found to be important, the optimum temperature being 5° to 12°C. Homogenized cream of 20 per cent butterfat would not whip. Fair whipping quality was observed in sterile creams of 25 to 35 per cent fat homoge-

nized up to 2000 pounds pressure, but the resulting product showed but little increase in volume, although a very stiff whip could be obtained. Creams having higher percentages of fat (30 to 35 per cent, homogenized at 2500 pounds) showed great separation of the fat after a year of storage, but readily mixed and their whipping properties were only slightly decreased as a result of storage.

<div style="text-align: center;">DISCUSSION</div>

The preheating of a good quality cream containing 20 per cent butterfat to 80°C. before homogenization allows the product to be sterilized with no coagulation. The cooked flavor of such a cream is not excessive and when eaten with fruit or cereal or in coffee would often be unnoticed. This cooked flavor is not so pronounced as that of evaporated milk, due probably to the much smaller percentage of solids not fat. After a considerable period of storage however, a slight "milk powder" or "old cream" flavor develops.

There is no marked or objectionable change in color or viscosity of the product during preparation or storage.

It is believed that an attractively packaged, sweet, sterile, cream would have excellent market possibilities where fresh cream is not available or where it is desirable to keep on hand a good quality cream for use on various occasions.

The possibilities of preparing a sterile whipping cream are less encouraging. Cream of 20 per cent butterfat will not whip easily, due to the high pressure of homogenization necessary to prevent separation. A sterile cream of a higher percentage of fat can be prepared but it is not entirely satisfactory because of the tendency of the fat to separate and the small increase in volume observed after whipping.

Optimum conditions for the preparation of a sterile whipping cream were attained when 30 per cent cream was preheated to 80°C., homogenized at 2500 pounds pressure, and sterilized at 118°C. for 12 minutes. This cream whipped well at 10°C. but with only a small increase in volume. A noticeable separation and an old cream flavor resulted after a year of storage of this product, although its whipping ability was still retained.

SUMMARY

The important factors in the preparation of a sweet, sterile cream have been discussed. Such a product may best be prepared by preheating a cream containing 20 per cent butterfat to 80°C., homogenizing at 3000 pounds' pressure, cooling, placing in bottles of the soda water type, and sterilizing at 118°C. for 12 to 14 minutes. This product will not whip easily. It will keep indefinitely without a seriously objectionable change in flavor or separation of the butterfat.

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THE FEED REQUIREMENTS AND THE FEED COST OF THE DAIRY SIRE*

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One of the necessary expenses of maintaining a dairy herd is the herd sire. The census of 1920 showed a total of 772,320 bulls one year and older in the United States. Apparently about one farmer out of six having dairy cattle keeps a bull. Unquestionably one reason for the widespread use of inferior sires is the considerable expense involved in the purchase and maintenance of a first class animal.

The purpose of the investigation reported was to determine: (1) the feed cost of keeping a bull under farm conditions and (2) to ascertain the amount of nutrients required to keep a bull in proper breeding condition.

The literature dealing with dairy management presents but little in the way of data concerning the feed requirements of the bull. The recommendations regarding the character of the ration are mostly in generalities. The usual recommendations are to feed sufficiently liberal to keep the sire in a vigorous condition but not too fat. Leguminous roughage is commonly recommended to be fed at the rate of 10 to 15 pounds daily with an allowance of corn silage not to exceed 15 pounds daily. The feeding of concentrates to the amount of from 4 to 8 pounds is generally suggested. A typical grain mixture selected from the various ones suggested is corn or barley 300 pounds, oats or bran 200 pounds and linseed oilmeal or other high protein concentrate 100 pounds.

Valuable data are available taken by the United States Department of Agriculture (1) on dairy farms in the states of Vermont, Indiana, Washington, and Louisiana. The records obtained were part of those taken in connection with a study of the unit

requirements for producing market milk in the regions indicated. Each farm was visited at monthly intervals by an agent who obtained the data required. For obvious reasons no money values are given for the feeds used or for the labor. A calculation based upon current prices will give this information if desired. A summary of the results is given in table 1.

THE COST OF FEEDING HERD SIRES ON MINNESOTA FARMS

The cost of feeding herd sires on dairy farms located in one of the older and better established dairy sections of Minnesota has been compiled from data secured from a statistical route operated

TABLE 1

Feed used and cost of keeping a bull for a year

	VERMONT	INDIANA	WASHINGTON	LOUISIANA	WEIGHTED AVERAGE
Number of animals................	27	33	34	24	
Concentrates, pounds	336	1,399	630	1,202	894
Dry roughages, pounds............	6,734	4,025	5,967	779	4,544
Succulent roughages, pounds	2,394	6,002	3,069	1,126	3,339
Bedding, pounds....	369	645	43	4	255
Labor, hours......................	37.7	35.7	40.4	33.6	37.08
Pasture...........................	$1.92	$4.56	$13.56	$4.66	$6.57
Total other costs*................	$14.21	$29.71	$41.81	$23.40	$28.37

* "Total other costs" includes interest, insurance, bull's share of buildings, depreciation, etc.

under the supervision of the Division of Agronomy and Farm Management of the University of Minnesota. The object of this route was to collect dependable information on the cost of producing farm products. A state and federal employee visited each farm approximately three times each week and recorded the desired data including the feed fed each animal and established a monthly market price for each feed based on local prices. Yearly feed records taken in this manner are available on 58 mature sires.

The average feed consumption is shown in table 2. Both roughage and concentrates were fed throughout the year. Little

difference is observed between the amount of dry roughage and concentrates consumed during the winter and summer months, the average being approximately 15 pounds of roughage and 4.5 pounds of concentrates per day.   As is to be expected the silage feeding was heaviest during the winter months and was generally discontinued in the month of April or May and resumed in late November or December.   This table further shows that some of the sires were on pasture during the summer months; the records show that 12 of the 58 sires were so handled.

TABLE 2

Yearly feed consumption per sire

Records from 58 animals

MONTH	DRY ROUGHAGE	CORN SILAGE	CONCEN-TRATES	PASTURE	TOTAL FEED COST
	pounds	*pounds*	*pounds*	*days*	*dollars*
January	455	637	137	0	6.30
February...........................	381	582	123	0	5.88
March	412	585	140	0	6.40
April	405	605	108	0	5.80
May	418	322	130	4.6	5 69
June	361	15	138	6.2	4.96
July.	421	95	92	5.3	4 67
August	443	106	100	5.3	5.10
September	496	25	156	3.6	5.86
October	477	135	179	2.7	6 24
November	564	251	124	0.5	6 19
December	553	644	140	0	6 62
Total..	5,386	4,002	1,567	28.2	69.71

The average grain ration fed, 1567 pounds per animal included corn 127 pounds, small grains 1375 pounds, commercial feeds 47 pounds and linseed oilmeal 18 pounds.   Corn, commercial feeds and oilmeal were fed sparingly while the small grains, which were largely oats and barley constituted most of the concentrates. The dry roughage fed the 58 animals included alfalfa, mixed, and prairie hay and corn fodder.   In a few cases a limited amount of straw was also used.   The value of the feed at current local market was $69.71 per year, although fluctuations due to season

and locality necessarily minimize the value of these figures. If the other expenses incurred in keeping these bulls are assumed to be $28.37, the same as found as an average for the 118 animals included in the study by the United States Department of Agriculture the total average expense involved in maintaining these bulls was $98.08 yearly. In addition about 37 hours of man labor are necessary which may be added at current rates to obtain the total cost involved.

THE SIRE AS A FACTOR IN THE COST OF MILK PRODUCTION

Sire cost is clearly a factor of some importance in the maintenance of dairy herds. The figures given show that the cost of keeping a bull in recent years has been approximately $100.00 per year in addition to labor. In some cases there is an appreciation in value of the animal as the result of growth or an income from service fees. At most, these possibilities will seldom reduce materially the expense incurred by the bull.

Where the herd is of typical size—10 to 15 cows, it is evident sire service becomes an important factor in the expense of raising heifers to maintain the herd. Assuming an 80 per cent calf crop, a herd of 15 cows will produce 6 heifers a year. For this number the sire cost will be $16.60 each. Unquestionably this sum is fully justified if the heifers raised have the heredity to become profitable dairy animals. The surprisingly high figure undoubtedly explains in part the reluctance of many dairy farmers to make use of improved sires. It also emphasizes the value of bull associations which make possible the use of high class sires at a minimum expense.

FEED REQUIREMENTS OF BULLS OWNED BY THE UNIVERSITY OF MINNESOTA

Feed records were kept of six mature bulls in the University herd. Weights were taken three days each month. The rations fed included alfalfa hay, corn silage and a grain mixture made up by weight of 200 pounds of ground oats, 100 pounds of ground corn and 100 pounds of ground barley. For a short period during

the summer green feeds were fed including sweet clover, oats and peas and fodder corn. A limited amount of mangels were also fed during a brief period. No attempt was made to feed the animals according to any feeding standard. They were given as much hay as they would clean up, silage to the amount of approximately ten pounds daily, and sufficient grain to keep them in what was considered good breeding condition. The changes in weight were slight during the experimental periods; in fact the weights at the beginning and end of the experimental periods show no wider range than variations from day to day with the same animals.

In table 3 are given the totals for the feed consumed during the experimental periods. Space will not allow more detailed presentation. The following statement of typical rations for summer and winter will supply some details that may be of interest. When green feed was used it replaced the silage.

Typical daily rations

	ALFALFA HAY	CORN SILAGE	GRAIN
	pounds	*pounds*	*pounds*
Winter:			
Jerseys	12	8	4 5
Holsteins	18	14	5.0
Ayrshires	16	12	5.0
Summer:			
Jerseys	12	5	5 0
Holsteins.....	14	13	5.0
Ayrshires...	12	12	5 0

The cost of the feed is not given in detail on account of the limited value of such figures due to local and seasonal variations. It may be said however that at local prices the average feed cost was approximately the same as that found in the cost accounting routes, data from which are given in table 2.

The digestible crude protein and total digestible nutrients received in these rations were calculated by using average figures for the feeds included as given by Henry and Morrison (2a). The results are given in table 4, and compared with the estimated

maintenance requirements of animals of the weights represented calculated according to the figures given by the same authors (2b).

TABLE 3

Feed consumed by mature bulls

ANIMAL	LENGTH OF PERIOD	WEIGHT	ALFALFA HAY	CORN SILAGE	GRAIN	GREEN FEED
	days	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Jersey A............................	365	1,330	3,673	753	1,652	2,900
Jersey B............................	365	1,250	4,575	3,647	1,487	
Jersey C............................	90	1,452	1,277	910	290	
Holstein A	365	2,510	6,550	3,420	2,146	1,891
Holstein B......................	153	2,310	2,494	1,836	687	
Ayrshire A......................	365	1.910	5.294	3.414	1.785	1.518

TABLE 4

Digestible nutrients consumed daily compared to maintenance requirement according to feeding standard

	WEIGHT	NUTRIENTS RECEIVED		MAINTENANCE REQUIREMENT BY FEEDING STANDARD		TOTAL DIGESTIBLE NUTRIENTS RECEIVED COMPARED TO FEEDING STANDARD
		Digestible crude protein	Total digestible nutrients	Digestible crude protein	Total digestible nutrients	
		pounds	*pounds*	*pounds*	*pounds*	*per cent*
Jersey A..................	1,330	1.64	9.9	0.93	10.5	91
Jersey B..................	1,250	1.81	11.3	0.87	9.9	114
Jersey C..................	1,452	1.90	11.5	1.02	11.5	100
Holstein A...............	2,510	2.67	16.0	1.76	19.9	80
Holstein B...............	2,310	2.26	13.9	1.62	18.3	76
Ayrshire A...............	1,910	1.77	14.0	1.34	15.1	92

It will be noted that four of the six bulls required less total digestible nutrients than the amount estimated from the feeding standard. Only one used more than the estimated nutrients. Thus results indicate that the bull offers no special problems so far as feed requirements are concerned. There is indeed a sug-

gestion of a maintenance requirement slightly lower than that ordinarily assumed for cows. The number of animals is too limited to justify any definite conclusions to that effect. It seems safe to conclude from the data that the ordinary maintenance requirements for total digestible nutrients may be assumed in connection with the feeding of mature bulls. The data gives no basis for estimating the protein requirements of the animal or the possible influence of the character of the ration upon the breeding qualities of the animal.

RATIONS FOR GROWING BULLS

The cost of raising a bull to maturity is a question of some importance. Although our records do not furnish complete data on this subject they do furnish some facts worth recording. Records were kept of the feed received and the gains in weights of four bulls representing four breeds, and between the age of one and four years. The ration used for these growing animals was the same in character as that supplied the mature animals. They were allowed all the roughage they would consume and in addition it was necessary to give them a liberal grain allowance in order to obtain what was considered a normal rate of growth. Two of them were fed more grain per day than the mature bulls received. The animals included were as follows:

	AGE AT BEGINNING		LENGTH OF PERIOD	WEIGHT AT BEGINNING	GAIN PER DAY
	years	months	days	pounds	pounds
Guernsey A	2	5	365	1,285	0 80
Guernsey B.		8	92	334	1 20
Jersey D		10	92	539	1 30
Ayrshire B..		10	212	448	1 52

The average feed received per day is given in table 5.

It is of interest to compare the nutrients received with the allowance that would be made by Morrison's feeding standard (2c). The weight of the larger Guernsey was beyond the range of the Morrison table and is for this reason omitted.

Nutrients received by growing bulls in percentage of Morrison's feeding standard

	DIGESTIBLE CRUDE PROTEINS	TOTAL DIGESTIBLE NUTRIENTS
Guernsey B..	102	103
Jersey D..	117	101
Ayrshire B...	98	85

These figures although few in number indicate that bulls fed according to accepted practices in well-managed dairy herds will ordinarily receive approximately the allowance of nutrients called for by a widely used feeding standard.

TABLE 5

Feed received per day by growing bulls

	WEIGHT	ALFALFA HAY	CORN SILAGE	GRAIN	GREEN FEED
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
Guernsey A........................	1,285	16.6	8.3	6.5	6.7
Guernsey B........................	334	5.4	4.0	4.0	0
Jersey D...........................	539	7.7	5.9	5.0	0
Ayrshire B........................	448	6.7	5.8	4.3	0

SUMMARY

A compilation from figures published by the United States Department of Agriculture gives the average feed received by 118 bulls on farms in four sections of the United States. In addition 37 hours man-labor were required, pasture to the amount of $6.57, and other cost totaling $28.37.

A summary is given of the feed received by 58 bulls on Minnesota farms over a period of a year. The average feed cost at current local prices was $69.71. Assuming the same other costs as found by the United States Department of Agriculture, the total cost of keeping a mature bull is approximately $98.00 a year in addition to about 37 hours of man labor.

The amount of feed consumed by six mature bulls in the University of Minnesota herd is given. The total digestible

nutrients contained approximate closely the maintenance requirements as estimated by the use of feeding standards.

The nutrients received by growing bulls fed according to common practice on dairy farms were close to the amounts prescribed by Morrison's feeding standard for growing dairy cattle of the weights represented.

REFERENCES

(1) BAIN, J. B., AND ASSOCIATES: United States Department of Agriculture, Bulletins 858, 919, 923, 955.
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NORMAL VARIATIONS IN THE INORGANIC PHOS-
PHORUS OF THE BLOOD OF DAIRY CATTLE*

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The inorganic phosphate of the blood has been studied ex-
tensively in recent years because of its relation to various prob-
lems of nutrition, metabolism, and disease. In cattle the effect
of the phosphorus level of the food on the percentage of inorganic
phosphorus of the blood has already been demonstrated by us (1)
(2). During the collection of these data we were impressed by
the fact that unaccountable, and frequently large fluctuations
may occur from day to day. This result led us to adopt the mean
of the blood phosphate values obtained on three consecutive
days as the probable true value. The day-to-day fluctuations,
however, remained unexplained.

The physiological factors influencing the concentration of blood
phosphate have not been studied extensively. Havard and Reay
(3) seem to have been the only workers who have noted marked
periodic fluctuations. They report that the concentration of
blood phosphate is very unsteady in human blood studied at
hourly intervals when the subject is not kept completely at rest.
Ordinary movements of the subject about the laboratory cause
large variations. Havard and Reay believe that these changes
are the result of exercise which they later (4) showed causes first a
small rise and then a rapid fall in blood phosphate. In these
studies the lowest level was reached in about three-quarters of an
hour after the period of exercise. Harrop and Benedict (5),
working with rabbits, observed that convulsions following

strychnine administration resulted in great increase in blood phosphate. Short, vigorous exercise causes an increase in blood pressure, according to the work of Young and coworkers (6). Scheunert and Müller (7) found with horses that vigorous running causes a marked loss in CO_2-combining capacity. The effects noted on blood pressure and CO_2-combining capacity may be coincident with, rather than related to the effects on blood phosphate.

A number of workers (5) (8) (9) (10) have noted that ingestion of soluble carbohydrates or the administration of insulin causes a decrease in blood phosphate. These results establish the intimate association between blood phosphate and glucose metabolism and support the belief (4) (5) that the effect of exercise on blood phosphate is directly related to this association.

The relation of blood dilution to phosphate concentration must be considered. Although no studies seem to have been made on the inorganic phosphorus, Priestley (11) found that water drinking under controlled conditions causes a slight but definite decrease in chlorides and dry matter. Greene and Rowntree (12) administered excessive amounts of water to dogs and secured dilution of the blood. Marx (13) found a marked dilution of the hemoglobin within 15 to 20 minutes after a man had drunk two liters of water or tea.

Meigs and Blatherwick (14) noted that the inorganic phosphorus is likely to be low in mature cows just after calving. They conclude that it seems to depend to some extent on the amount of grain fed. Robinson and Huffman (15) in an extensive study found that in nearly every case, the inorganic phosphorus of the blood of the dam is below that of the calf at birth, but that the values for the maternal inorganic phosphorus usually rise during six to eighteen hours after calving to a rather definite maximum which is followed by a depression. Beyond this the fluctuations are irregular. In their work with calves, Meigs, Blatherwick, and Cary (16) found the inorganic phosphorus of the plasma to be fairly high in new born calves but tending to increase for some time and reaching a maximum at about the age of six months.

The experimental results fall into two groups. The first group comprises the normal variations in blood phosphate from day to day in samples taken at the same hour on three consecutive days. Some variations encountered at periodic intervals on the same day are also reported. The second group consists of the results of attempts to determine the extent to which such physiological factors as eating, drinking, and exercise affect the blood phosphate of cattle. It was hoped that these factors would throw light on the daily variations encountered. In addition, data are presented on the influence of parturition and age on the inorganic phosphate in the blood.

The blood used in the analyses was drawn from the jugular vein, and allowed to flow into 100 cc. glass tubes, containing 1 cc. of a saturated solution of sodium citrate. The size of sample drawn was 50 cc. The blood was mixed, thoroughly, with the sodium citrate solution immediately to prevent coagulation. It was then centrifuged to separate the plasma from the corpuscles. Upon completion of the centrifugation, sufficient plasma for the test was pipetted off, and preserved in an ice box until tested. In nearly all cases the analyses involving single samples were completed the same day the blood sample was drawn. Where the nature of the study permitted, the test was made on a composite sample of plasma taken from blood drawn at the same hour on three consecutive days. In some of the work in the second group of experiments, however, composite samples could not be used, and individual samples of plasma were analyzed for inorganic phosphorus.

Method of analysis

The Briggs (17) modification of the Bell-Doisy colorimetric method of phosphate analysis was used. All tests were made in duplicate and the averages were used in all comparisons.

Animals used

Twenty-five different animals furnished the data for the first group. They were all in the University experimental herd.

TABLE 1

Day-to-day variations in inorganic phosphate in successive three-day periods per 100 cc. plasma

ANIMAL	PERIOD 1 P1 a	P1 b	P2 a	P2 b	P3 a	P3 b	PERIOD 2 P1 a	P1 b	P2 a	P2 b	P3 a	P3 b	PERIOD 3 P1 a	P1 b	P2 a	P2 b	P3 a	P3 b	PERIOD 4 P1 a	P1 b	P2 a	P2 b	P3 a	P3 b
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
E 33	3.06	3.12	2.37	2.54	2.07	2.12	2.55	2.57	1.96	1.98	2.08	2.08	2.31	2.31	3.33	3.37	2.93	2.93	1.74	1.89	1.67	1.67	1.75	1.75
E 73	2.26	2.37	2.72	2.76	2.28	2.40	2.40	2.42	2.44	2.51	2.01	2.02	2.43	2.45	1.92	1.92	1.92	1.92	2.35	2.35	1.71		1.90	
E 74	5.04	5.04	5.74	5.88	5.65	5.71	4.25	4.25	5.55	5.74	5.74		4.95	4.95	2.65	2.65	4.35	4.35	4.80	4.80	4.64	4.67	4.80	4.80
E 75	1.72	1.79	1.82	1.86	1.59	1.72	1.79	1.79	1.84	1.97	1.63	1.63	1.90	1.93	2.12	2.31	1.69	1.71	2.45	2.56	2.14	2.24	2.39	2.50
E 91	2.29	2.33	2.24	2.29	2.51	2.63	1.77	1.85	1.94	1.95	2.04	2.04	1.00	1.01	5.21	5.21	2.51	2.60	0.88	0.88	1.17	1.17	1.06	1.06
E 92	3.79	4.03	4.27	4.35	5.00	5.00	3.70	3.70	3.85	3.95	3.33	3.35	5.20	5.20	8.34	8.75	5.25	5.26	0.88	0.88	1.17	1.17	4.86	4.89
E 93	1.07	1.09	0.93	0.95	1.20	1.40	2.12	2.12	1.97	2.02	1.91	1.96	1.96	1.00	1.23	1.24	8.90	9.11	1.53	1.53	1.90	2.00	1.25	1.29
E 94	1.29	1.31	1.13	1.14	1.40	1.40	2.27	2.27	2.17	2.17	1.56	1.56	1.73	1.77	1.27	2.90	9.10	9.2	1.21	1.210	840		1.25	1.29
E 84	9.09	9.25	7.14	7.24	7.09	7.09	4.99	4.99	4.95	4.95	5.37	5.37	6.06	6.06	2.16	2.56	5.26	61						
E 58	7.04	7.14	6.91	7.04	5.54	5.67	4.11	4.15	4.35	4.36	6.75	6.75	5	4.95	2.65	2.65	5.26	61						
E 68	6.62	6.71	6.75	6.85	6.57	6.15	5.67	5.67	6.45	6.56	7.04	7.04	6.06	066	2.16	2.56								
E 77	8.07	8.19	8.13	8.13	8.77	8.93	7.09	7.09	6.90	6.90	2.16	25												
E 80	7.04	7.24	6.85	6.85	6.85	6.85	6.75	6.75	6.75	6.75	2.56	49												
E 81	9.43	9.52	8.00	8.06	6.67	8.06	7.14	7.14	7.07	7.07	7.307	40												
E 83	7.46	7.57	6.25	6.45	6.67	6.17	7.14	7.14	7.07	7.07	097	19												
E 85	9.52	9.90	7.24	7.24	8.06	8.26	7.99	7.99	7.14	7.147	407	46												
E 86	8.13	8.13	7.75	7.87	7.63	7.63	7.30	7.307	7.45	7.457	576	57												
E 88	9.52	9.52	7.30	7.40	8.06	8.13	8.93	9.37	7.517	517	268	26												
E 89	8.33	8.33	7.57	7.93	7.81	7.81	8.17	8.18	7.566	806	90													
E 78	7.69	7.80	6.45	6.45	6.17	6.33	8.07	807	457															
E 79	6.90	6.90	6.90	6.90	6.49	6.49																		
E 87	8.93	9.08	8.48	8.62	10.41	10.63																		
E 95	7.34	7.45	6.40	6.40	7.51	7.75																		
E 96	9.70	9.89	8.48	8.48	8.61	8.76																		

The ages of the animals ranged from calves to mature cows, some of the latter being in milk. The animals represented various planes of phosphorus intake.

All the animals used in the second group, with the exception of two cows, were from the general University herd, and were being fed the usual herd rations. The two cows excepted were from the University experimental herd. They were both receiving either normal rations or a phosphorus-deficient ration plus a phosphate supplement. Yearling heifers fed normal rations were used for the study of the feeding and drinking factors. In the study of the age factor, calves from the general herd consisting bf Holstein-Friesian, Jersey, and Guernsey breeds were used. Their feed while young consisted of milk, but alfalfa hay and cracked corn were given as soon as they would eat them.

DAY-TO-DAY VARIATIONS

The data collected comprise 60 sets of three-day samples, taken at monthly intervals from August to December, 1926. Five animals had only one period of 3 consecutive days, 10 animals had 2 periods, 1 animal 3 periods, and 8 animals 4 periods. The complete data, including the duplicate analyses of each sample are given in table 1. In this table P is the milligrams of inorganic phosphorus per 100 cc. of plasma, the subscripts 1, 2, and 3 referring to the first, second, añd third days, and the subscripts a and b to the duplicate analyses.

The coefficients of correlation between the respective days, calculated by the formula of Harris (18) are as follows:

$$r_{P_1P_2} = +0.970033 \pm 0.005141$$
$$r_{P_1P_3} = +0.930870 \pm 0.011623$$
$$r_{P_2P_3} = +0.954174 \pm 0.007798$$

It is apparent that there is a high correlation between the values obtained on successive days when the data are considered as a whole. However, simple inspection of the data in table 1 shows that marked variations from the general law are of frequent occurrence. In order to secure data showing to what extent the actual values deviate from the theoretical values indicated
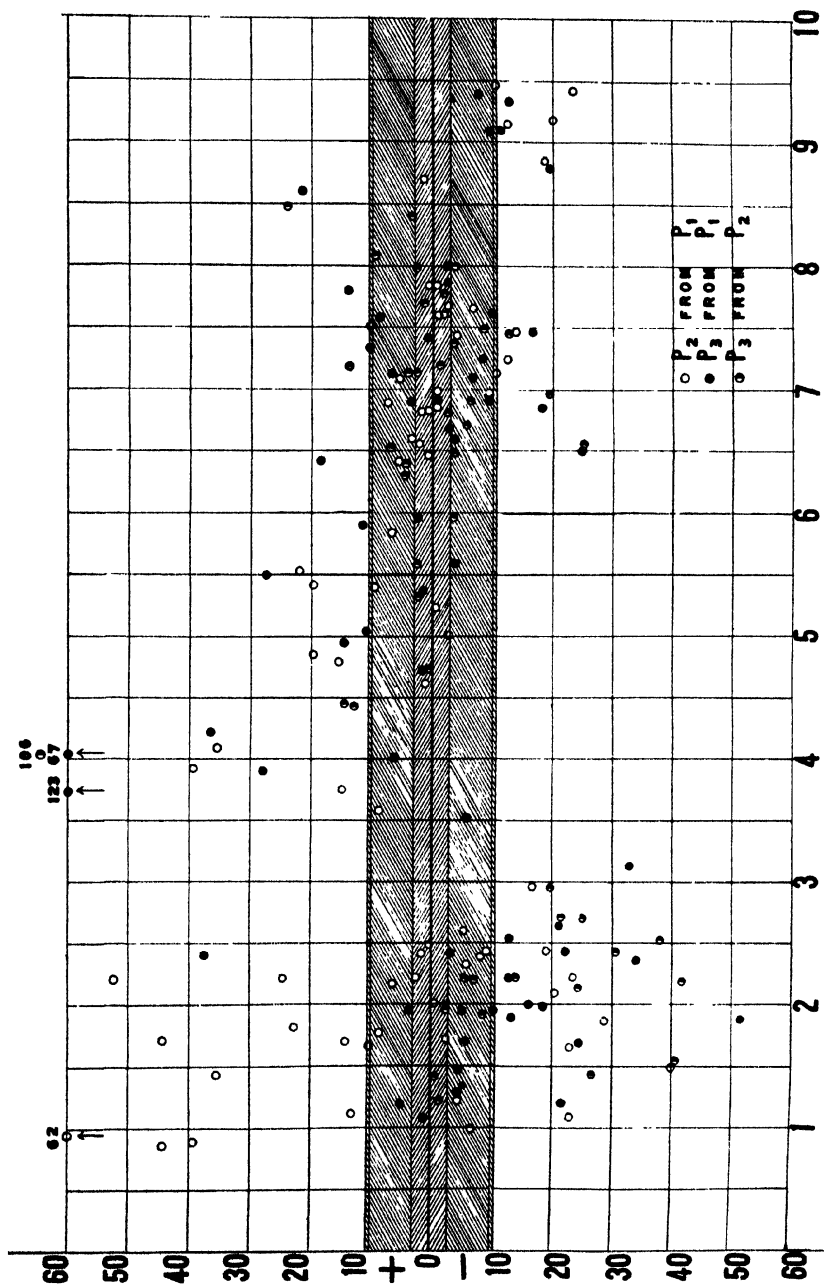
FIG. 1

by the coefficient of correlation, use was made of Harris' modification of the regression formulae, as follows:

(1) $\qquad P_2 = (\bar{P}_2 - r_{P_1P_2}\bar{P}_1) + r_{P_1P_2}P_1$

(2) $\qquad P_3 = (\bar{P}_3^j - r_{P_1P_3}\bar{P}_1^j) + r_{P_1P_3}P_1$

(3) $\qquad P_3 = (\bar{P}_3 - r_{P_2P_3}\bar{P}_2) + r_{P_2P_3}P_2$

Thus, by formula (1) it is possible to calculate the theoretical value of P_2 using P_1, the mean values of P_1 and P_2 of the entire series, and the coefficient of correlation between P_1 and P_2. Similarly, formula (2) makes it possible to calculate P_3 from P_1, and formula (3) makes possible the calculation of P_3 from P_2. The calculation is a simple one, inasmuch as the parenthetical value in each equation is a constant for the equation, so that formulae (1), (2), and (3) become

(1) $\qquad P_2 = K_1 + r_{P_1P_2}P_1$

(2) $\qquad P_3 = K_2 + r_{P_1P_3}P_1$

(3) $\qquad P_3 = K_3 + r_{P_2P_3}P_2$

Using the calculated value for K, the formulae become

(1) $\qquad P_2 = -0.042 + 0.970\ P_1$

(2) $\qquad P_3 = +0.256 + 0.931\ P_1$

(3) $\qquad P_3 = +0.322 + 0.954\ P_2$

Figure 1 shows the percentage variations of the actual values from the theoretical values obtained from equations (1), (2), and (3). The physiological significance of these variations is revealed by the points outside of and within the cross-hatch lines. The lines drawn at $+10.37$ and -10.37 per cent represent the maximum analytical error of the duplicate determinations on the same sample of plasma. All the points outside these lines are therefore physiologically significant. The lines drawn at $+3$ and -3 per cent were arbitrarily chosen because calculation of the analytical errors showed that 85 per cent of the mean values of duplicate analyses had an error of 3 per cent or less. This means that 85 per cent of the points between the 3 per cent and the 10.37 per cent lines represent physiologically significant variations. In like manner 15 per cent of the points between the 0 line and the 3 per cent lines are significant variations. A sum-

mation of the significant variations of the actual from the theoretical values shows a total of 137.55 out of 180, or 76.4 per cent.

It was the striking day-to-day variations of the inorganic phosphate in the blood of single cows that prompted the major portion of the second group of experiments. It seems probable that the inorganic phosphorus content of the blood plasma of cows reported in the literature, which apparently represent single random samples, are very likely incorrect. In fact, our data indicate that there is only about one chance in four of such a procedure giving a correct result.

TABLE 2

Inorganic blood phosphate variations at two-hour intervals during the day

| TIME | COW NUMBER | P PER 100 CC. PLASMA | |
		(a)	(b)
		mgm.	*mgm.*
9:00 a.m.	E 75	5 15	5.15
11:00 a.m.	E 75	5.10	5 05
1:00 p.m.	E 75	4.16	
3:00 p.m.	E 75	4 16	4 24
5:00 p.m.	E 75	5.10	5 10
9:00 a.m.	E 73	1.41	1 41
11:00 a.m.	E 73	1.98	1 96
1:00 p.m.	E 73	1 96	1 96
3:00 p.m.	E 73	1.52	1.54
5:00 p.m.	E 73	1.60	1 56

Numerous physiological factors might be responsible for these variations, most of which are impractical to control experimentally. Their influence cannot be determined at present.

Blood was drawn from two dry cows at two-hour intervals, beginning at 9:00 a.m., to determine whether the inorganic phosphorus varies appreciably at such intervals. Each cow was in her stall and allowed water *ad libitum* but was not fed. The results, given in table 2, show a marked depression followed by a rise in the case of one animal and a moderate rise followed by a depression in the case of the other animal. These variations resemble those found by Havard and Reay (3) to occur in humans.

The three physiological factors which can be readily controlled experimentally and which from the review of the literature, may be expected to exert an influence on the percentage of inorganic phosphorus of the blood are eating, drinking, and exercise. The latter might be especially significant in the case of animals that struggle violently before the blood sample can be taken.

TABLE 3

Effect of feeding on inorganic phosphate in blood plasma of cattle

COW NUMBER	PLASMA P BEFORE FEEDING	CHANGES IN PLASMA P			
		Three-fourths hour after feeding	One and three-fourths hours after feeding	Two and three-fourths hours after feeding	Five hours after feeding
	mgm. per 100 cc	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
402	6 41	+0.68*		−0.42*	−0.50*
402	6.49	+0.80*	+0.08	−0 59*	
402	5 83	+0.46*	+0.08	−0.12	
403	7.14	−0.10		−0.02	+0.17*
403	8.13	+0 49*	−0.07	−0.49*	
403	7.04	+0.31*	+0 23*	−0.28*	
175	6 54	+0.08	+0 40*	−0 22*	
176	6.94	+0.52*	−0.11	−0.31*	
176	6.49	−0 28*	+0.18*	−0.80*	
174	6 62	−0 13	−0.08	−0.26*	

Difference between duplicate analyses

	mgm.
Mean.	0.043
Maximum	0.14
Minimum.	0 00

* Significant change.

INFLUENCE OF FEEDING

Ten trials were run to study the effect of feeding on the inorganic blood phosphate. Blood samples were drawn before feeding in the morning and again at intervals of 45 minutes, 1.75 hours, and 2.75 hours, and in 2 cases 5 hours after feeding. The animals were kept in the stall continuously both prior to and after feeding. Water was allowed *ad libitum* from drinking fountains in the stalls. The feed was the same as used in the herd for growing heifers and was considered a normal ration.

Table 3 gives the results of the various trials and shows the inorganic plasma phosphate before feeding and the consecutive milligram changes at the various intervals after feeding. The significance of the changes has been interpreted after subtracting the maximum analytical difference obtained in duplicate analyses of the same sample. This is the most rigid interpretation possible. On this basis it is seen that a significant rise in blood phosphate had taken place by three-fourths of an hour after feeding in 6 of the 10 trials, and a significant fall in one trial, with no significant change in the other 3 trials. In one of the latter trials, a significant rise had occurred by the end of 1.75 hours, and in another there was a significant fall which was not manifested until the end of 2.75 hours.

There are not enough significant changes at the end of 1.75 hours to indicate any single physiological effect. At the end of 2.75 hours, however, a significant fall had occurred in eight of the ten trials in comparison with the inorganic blood phosphate the previous hour.

The mean magnitude of significant change in blood phosphate at the end of the 0.75 hour interval over and above the maximum analytical difference amounts to 0.363 mgm. or 5.7 per cent of the mean blood phosphate in these cases. This is not a great effect but is sufficient to warrant consideration in determining a procedure for taking blood samples from dairy cattle.

INFLUENCE OF DRINKING

Six trials were run to study the effect of drinking by cattle on the inorganic phosphorus content of the blood. The animals were first deprived of water, for various lengths of time in the different trials, to insure the drinking of water at the time it was desired to conduct the test. In the first and second trials, the animals were deprived of water for 24 hours, in the third and fourth trials for 30 hours, and in the fifth and sixth trials, they were deprived of water for 44 hours to insure copious drinking. Blood samples were drawn in each case just before the animals were watered, and again at 2 thirty-minute intervals after they had finished drinking. As shown in table 4, there was a decrease

in blood phosphate in every case 30 minutes after drinking. In only 2 cases, however, was there any further decrease one hour after drinking.

The maximum analytical error in these analyses was the same as in the experiments on the effect of feeding, namely, 0.14 mgm. Applying this figure in the same manner as in the experiments on the effects of feeding shows that 5 of the 6 trials give a significant fall in blood phosphate after drinking. However, the mean percentage variation of the significant change is small, amounting

TABLE 4

Effect of water drinking on the inorganic phosphate in blood plasma of cattle

COW NUMBER	TIME DEPRIVED OF WATER	WATER DRUNK	PLASMA P BEFORE DRINKING	CHANGE IN PLASMA P	
				One-half hour after drinking	One hour after drinking
	hours	*pounds*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
402	24		7.14	−0.10	0.00
402	30	40	5.57	−0.33*	+0.14
402	40	59	6.25	−0 52*	+0.15
403	24		8.77	−0.30*	−0.78*
403	30	43	7.35	−0.38*	+0.17*
403	44	56	6.25	−0 26*	−0.46*

Difference between duplicate analyses

		mgm.
Mean	....	0 021
Maximum	... .	0.14
Minimum	....	0 00

* Significant change.

to only 3.6 per cent of the value of the blood phosphate before drinking. In view of the fact that the cattle in these experiments drank an excessive amount of water, it is unlikely that the ordinary amounts of water drunk by cattle at any one time will have any significant influence on the true content of inorganic phosphate.

INFLUENCE OF EXERCISE

Twelve trials were run to study the effects of exercise on the inorganic blood phosphorus. In the first 6 trials, heifers about

seven months old, which had a high blood phosphorus content, were used. In trials 7 to 10, inclusive, cows with a medium content of inorganic blood phosphorus were used, while in the eleventh and twelfth trials, yearling heifers were used.

In each case, the animal was bled after having eaten in the morning. It was then turned out into a lot and caused to run for 5 to 10 minutes or until it was breathing hard. It was then re-

TABLE 5

Effect of exercise on the inorganic phosphate in blood plasma of cattle

COW NUMBER	PLASMA P BEFORE EXERCISE	CHANGES IN PLASMA P			
		At once	One-half hour after exercise	One hour after exercise	Two hours after exercise
	mgm. per 100 cc.	*mgm per 100 cc.*	*mgm per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
179	8.77	+0.40*	−2.50*		
179	7.25	+0.44*	−0 84*	−0.18*	+0 58*
179	8.00	+0 47*	−1.48*	−0 54*	+0.09
554	8.66	−0 19*	−1.91*		
554	8 06	+0 07	−0.73*	+0 10	+0.30*
554	8.89	+0.28*	−1.11*	−0 19*	+0 13
75	4.26	+1 30*	−2 23*	−0 39*	+0 09
75	4.08	+0.14	−0.60*	−0.52*	−0 31*
93	4 06	+0.19*	−1.49*	+0.77*	−0 04
93	4.35	+0.75*	−1.40*	+0.65*	−0 26*
402	5 95	+0.75*	−1.08*	+0 12	+0.07
403	8.06	+0 71*	−0 77*	−1.01*	−0.19*

Difference between duplicate analyses

	mgm.
Mean	0 034
Maximum.	0 17
Minimum..	0 00

* Significant change.

turned to the stall and bled as quickly as possible. Usually, about fifteen minutes elapsed between the first and second bleedings. The animal was then allowed to stand quietly in the stall, and was re-bled at one-half-hour, one-hour, and two-hour intervals. In the first 2 trials, the one-hour and two-hour intervals were omitted.

The results of the study are given in table 5. They show a

marked increase of inorganic phosphate just after exercise, followed by a still more marked decrease at the half-hour, and in most cases a still further, but less marked decline after a further half-hour.

The relative significance of these changes after subtracting the maximum analytical difference between duplicate analyses is striking. Ten of the 12 trials gave a significant rise in inorganic phosphate immediately after the period of exercise, and in every trial a significant fall had occurred at the end of one-half-hour. In 6 out of 10 trials there was a further significant fall after another half-hour interval, while 2 trials gave a significant rise after this period. At the two-hour interval after the exercise apparently all but one of the animals had either attained the maximum after-effects of the exercise or had partially recovered. Animal 75 (second test), which apparently showed the after-effects of the exercise more slowly than the other animals still exhibited a further fall in blood phosphate at this time. The figure for animal 403 at the two-hour period is not greatly in excess of 0.17 mgm., the analytical error applied.

Viewed from a percentage standpoint the mean significant changes are as follows. Immediately after exercise there was a rise of 6.34 per cent compared with the value before exercise. One-half hour later there was a fall of 22 per cent compared with the value immediately after exercise. After another half-hour there was a further drop of 4.9 per cent, using the first half-hour value as a basis.

It is evident that the activity of the animal is a very significant factor in modifying the inorganic phosphate of the blood. Some of the variations occurring with individual cows were almost as great as some of the day-to-day variations encountered. For example, in the first test with animal 75 there was first a 30.5 per cent increase followed by a 40 per cent decrease. Apparently every possible precaution should be taken to avoid unnecessary activity in drawing blood samples from cattle for estimation of inorganic phosphate content.

INFLUENCE OF PARTURITION

Opportunity was presented to study the influence of 2 other normal physiological factors on inorganic blood phosphorus, namely, the effect of parturition and of age. Obviously it was not expected that such data would throw any light on the day-to-day variations.

TABLE 6

Changes occurring in the inorganic phosphorus content of the blood of cows at parturition

DAYS FROM CALVING	INORGANIC PHOSPHORUS IN 100 CC. OF PLASMA					
	No 75	No 81	No 93	No. 407	No 400	No 547
Before	mgm	mgm.	mgm.	mgm.	mgm.	mgm.
3 or more	4.79	4.40	4.16	5.32		4.84
2		4 89	5 43		4 50	4 95
1		3.07	3 51	4.56	4 31	2.77
0 (calved)	2.46	3.73	3.39	2.14	4 25	2.87
After						
1	2.64	4 22	3.83	2.17	3.33	2.69
2	2.81	4.74	5.10	4 07	2.51	2.72
3	4.92	5 36	3 79	4 17	3.47	3.70
4					3 40	
5		6.22	1.67	4 35		4.85
6	4 86				3.77	
7		5.84				
8			2.06	4.33		
9		6.49			4.08	
10						
11		5.92	4.25		5.70	

Six cows were used in the study of the effect of parturition. The general plan was to test a three-day composite sample of blood plasma, about one week before parturition, then take daily samples of blood so that individual samples could surely be tested on the 3 days immediately preceding calving. On account of certain cows calving sooner than expected, incomplete records were secured in those cases. In general, the results showed there was not much change in the inorganic phosphorus until less than

3 days before calving. Therefore, in order to simplify the tabulation of the results, all tests made 3 or more days before parturition were averaged, and this figure was taken as the starting point in the tables.

Table 6 shows the results of the study in tabular form. The amount of inorganic phosphorus in the blood on the day of calving is shown between the horizontal lines opposite "0" days. Most

TABLE 7

Comparison of the inorganic phosphorus content of the blood of the newborn calf with that of the dam

DAYS FROM CALVING	INORGANIC PHOSPHORUS IN 100 CC. OF PLASMA					
	No. 407	No. 407's calf	No. 409	No. 409's calf	No 547	No. 547's calf
Before	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
3 or more	5.32				4.84	
2	5.43		4.50		4 95	
1	4.56		4.31		2.77	
0	2.14	5.13	4.25	5.08	2.87	5 43
After						
1	2.17		3 33	6 25	2.69	
2	4.07	6.13	2.51	6.49	2.72	5.73
3	4.17	6.76	3.47		3.70	
4			3.40			
5	4.35				4.85	5 97
6			3.77	6.90		
7		6.90				6.10
8	4.33					
9			4.08			

of the cows showed a tendency to be low in phosphorus at the time of calving. In every case where comparative records were obtained, there was a decrease in inorganic phosphorus on the day before parturition. This decrease from the preceding day varied from 0.2 to about 2.2 mgm. of inorganic phosphorus per 100 cc. of plasma.

The time of lowest inorganic phosphorus in the blood was quite variable. In only 2 cases was it lowest on the day of calving. The other low points were observed on the day preceding par-

turition, on the day following and 2 days after calving, while one, No. 93, had a distinct decrease near the time of calving followed by a rise and then by a decrease much greater than the first, until on the fifth day from calving her blood contained only 1.67 mgm. of inorganic phosphorus per 100 cc. of plasma. This was about 2.5 mgm. below her average value three or more days before calving.

None of the other cows showed any tendency to have a second decrease several days after calving. No. 409, however, continued to decrease until 2 days after parturition when the inorganic phosphorus was 2 mgm. below the value shown 2 days before calving. The amount of decrease in inorganic phosphorus shown by the various cows at or near the time of parturition, varied from about 1.3 to 3.2 mgm. per 100 cc. of plasma. In only one case was the decrease less than 2 mgm.

Three calves were tested for inorganic blood phosphorus at or soon after birth, and at intervals during the first week. Table 7 shows the comparison of the calf blood with that of its dam. In every case, the blood of the calf contained more inorganic phosphorus at birth than that of the dam, and the value compared favorably with that of its mother several days before calving.

INFLUENCE OF AGE

The calves in the dairy herd, not over 185 days old, all of which were fed on normal rations, were used to study the effect of age on the inorganic phosphorus content of blood. These were classified so that they could be tested at or near their monthly birth dates. The limits of ages for testing were 0 to 7, 25 to 35, 55 to 65, 85 to 95, 115 to 125, 145 to 155, and 175 to 185 days. To make a comparison with animals of older ages, 26 tests, previously made, were tabulated. The older animals were not tested within any particular age limits. The system of age classification used for them was therefore different from that used for the calves under 185 days of age.

The results of this study are shown in table 8. The calves were distinctly lower in the inorganic phosphorus content of the blood during the first week after birth and increased fairly rapidly

up to 2 months of age, followed by a more or less variable period up to 4 months, when there was a gradual rise to 6 months of age. The average inorganic blood phosphorus content for all calves used, from 0 to 185 days old, was 7.26 mgm. of phosphorus per 100 cc. of plasma.

After 6 months, there began a slow, gradual decline in the blood phosphate. Calves at an average age of 274 days had a

TABLE 8

Effect of age on the inorganic phosphorus content of cattle blood

NUMBER OF ANIMALS	RANGE OF AGES	AVERAGE AGE	AVERAGE INORGANIC P IN 100 CC.
	days	*days*	*mgm.*
5	0 to 7	3	5.97
6	25 to 35	29	7.03
6	55 to . 65	59	7.57
6	85 to 95	91	7.19
7	115 to 125	121	7.53
7	145 to 155	149	7.68
9	175 to 185	180	7.89
Average	0 to 185	90	7.26
5	269 to 280	274	7.25
6	306 to 365	337	7.20
5	406 to 463	436	7.02
4	497 to 547	527	5.91
3	559 to 589	575	5.55
3	600 to 650 •	626	5.94
*Average.	Mature cattle		5.87

* The average given is one obtained by Robinson and Huffman (15).

mean inorganic phosphorus content of 7.25 mgm. per 100 cc. of plasma. At an average age of 436 days, the value had decreased to 7.02 mgm., and at 527 days there was an average blood phosphate content of 5.91 mgm. per 100 cc. of plasma. After this age the results were variable but with no pronounced change up to 626 days. At this time they had apparently reached approximately the normal value for mature cattle.

DISCUSSION OF RESULTS

The inorganic phosphate in cattle blood cannot be determined with accuracy by random sampling. It appears that wide fluctuations may occur from day to day in consecutive three-day tests even when the samples are taken under presumably identical physiological conditions. Although calculation from 60 such three-day series shows a very high coefficient of correlation between the consecutive samples, yet nearly 50 per cent of the individual samples varied 10 per cent or more from the regression line, a few showing over 100 per cent variation from the expected value. Of the 3 physiological factors studied, namely, exercise, food ingestion, and water drinking, only the first gave results of sufficient significance to account for the abnormally great fluctuations. Our study seems to show fairly definitely that vigorous exercise usually causes an increase in the inorganic blood phosphorus, followed within a half-hour by a marked decrease to a point below the "before exercise" level. The blood phosphate usually remains low for a period of at least 2 hours after exercise. The amount of increase observed in these trials seemed to depend on the severity of the exercise and upon getting the blood sample quickly after exercise ceased. It would appear that the increase is more or less transitory, but that the decrease which follows persists for several hours. The increase in inorganic blood phosphorus as a result of exercise is possibly caused by a breakdown in hexose phosphates, to supply energy, thereby releasing inorganic phosphorus into the blood. The decrease which follows is, by analogy, the result of a resynthesizing of hexose phosphates. It would seem that any severe resistance of an animal to the act of bleeding would cause changes in the inorganic phosphorus of the blood, but that in animals that submit meekly to this process, there would be no significant change.

A significant but small increase in blood phosphate appears to occur within the first hour of the normal food ingestion in most cattle. This may be great enough to account for day-to-day fluctuations of 5 to 10 per cent from the expected value in case no attempt is made to control the blood sampling with reference

to periods of time in close proximity to the normal period of feeding. In general, this rise seems to persist for about 2 hours after the food ingestion, which is followed within the next hour by a fall which brings the blood phosphate to approximately the original value before feeding. It would appear that bleeding time should be either before feeding or about three hours after feeding, if the effect of food ingestion on blood phosphate is to be avoided. It is doubtful whether food ingestion caused any of the day-to-day fluctuations in our observations, because the blood samples were all taken at the same hour each day, thus bringing them to the same proximity to the time of feeding.

The inorganic blood phosphate in cattle does not respond to food ingestion in the same manner as is produced by the ingestion of soluble carbohydrates by man and small animals. The depression noted by others to occur immediately after glucose ingestion does not occur in cattle until about three hours after feeding. Glucose from digested carbohydrates evidently does not reach its height of absorption until about three hours after food ingestion and even then the effects on inorganic blood phosphate through the formation of hexose phosphates are mild. The increases in inorganic phosphate occurring soon after eating may be due primarily to the release of phosphate from the tissues as a result of muscular activity accompanying the peristalsis of the extensive digestive system of this species.

The effect of water drinking on inorganic phosphate in cattle blood is negligible. The fluctuations noted were under 5 per cent even when the cattle were deprived of water as long as 44 hours. Under normal conditions one cannot attribute day-to-day fluctuations to this factor.

A marked decrease in the inorganic phosphorus content of cow's blood occurs at or near the time of parturition. The amount of decrease noted in this study varied from 1.3 to 3.2 mgm. of inorganic phosphorus per 100 cc. of plasma. The decrease sets in on the day before calving and usually persists for several days. The lowest point of decrease may occur either before or after parturition but usually within 2 days of that event. The reason for this marked decrease in blood phosphate at the time of par-

turition is not fully understood, but it is probably associated with some special metabolism taking place at that time. Since we know that phosphorus is associated with the metabolism of both fat and glucose, one would suspect that the rather sudden disappearance of much of the phosphates from the blood as a result of calving is associated with some rapid metabolism or storage of these compounds.

The inorganic phosphorus content of calf blood is much higher at birth than that of the dam. The level of phosphate content in calf blood at birth compares favorably with that of the dam's blood several days before calving. Evidently, the fetus is influenced in its blood phosphate content by the dam, but when the dam decreases in blood phosphorus at or before calving, the calf maintains a higher level. In the cases observed, the calves showed a marked increase in inorganic blood phosphorus content within 2 days after birth, showing that when on an independent diet they can quickly build up the blood phosphorus level.

During the first week after birth calves are lower in inorganic blood phosphorus than older calves, but higher than their dams. In the cases studied, they increased to a maximum at about 180 days of age. The average inorganic phosphorus content of blood at this age was 7.89 mgm. per 100 cc. of plasma. The average content of inorganic phosphorus in the blood of calves 0 to 185 days old was 7.26 mgm. per 100 cc. of plasma. After an average of about 180 days, the trend of blood phosphate seems to be downward. The number of animals used in this study was too small to make the results conclusive, but they indicate that calves ordinarily increase in blood phosphate until about six months of age, then gradually decrease until they reach the normal for mature cattle at about eighteen months old.

CONCLUSIONS

The inorganic blood phosphate in individual cattle may vary markedly from day to day even when the blood is drawn under apparently identical conditions.

The inorganic blood phosphate in individual cattle may vary

considerably from hour to hour, although the data available on this point are very limited.

Exercise causes marked changes in blood phosphate of cattle. There is first a definite rise, which is followed by a marked fall which persists for several hours.

Feeding has a small but significant effect on the inorganic phosphate in cattle blood. The value rises within the first hour and apparently does not return to normal until after about three hours.

Normal water drinking by cattle has no significant effect on blood phosphate.

So far as the factors studied are concerned, the procedure indicated for securing normal samples of blood from cattle are to have the animal at rest in its stanchion for several hours and draw the blood before feeding, with the least possible physical disturbance of the animal. Water should be allowed *ad libitum*.

Parturition causes a decrease in the inorganic blood phosphorus which may amount to as much as 3.2 mgm. per 100 cc. of plasma. The decrease sets in on the day before calving, the lowest point of decrease occurring either before or after parturition.

The phosphate content of calf blood at birth is higher than that of the dam, and compares favorably with the value shown by the dam several days before calving.

Calves increase in the inorganic phosphorus content of their blood until about six months old, after which a decrease sets in which continues until the normal range for mature cattle is attained.

The blood analyses shown in tables 1 and 2 were made by W. M. Neal, Special Analyst for the mineral nutrition project of the Experiment Station. Credit is due Professor J. Arthur Harris, Station Biometrician, for the formulae used in connection with the day-to-day variations, and to Miss Rachel Rude, Station Assistant, for the calculations.

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A STUDY OF THE RELATION BETWEEN THE TIME A COW IS CARRIED IN UTERO AND HER MATURE EQUIVALENT BUTTERFAT PRODUCTION*

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This study was originally suggested by Dr. W. L. Williams, Emeritus Professor of Cornell University, in personal correspondence to the authors in which he raised the question of the possibility of the producing efficiency of a cow being influenced by the duration of time she was carried in utero by her dam.   He goes on further to say,

first you will perhaps regard this as absurd, but in thoroughbred horses, V. Ottingen, stud-master at Trakehnen, one of the most famous imperial studs in Europe, has declared that a foal carried over 335 days in utero rarely distinguishes itself in later years and that a foal carried over 345 days virtually never proves satisfactory.   He submits considerable important evidence in support of his contention.

Prolonged gestation in the mare somewhat paradoxically appears to be the analogue of abbreviated pregnancy in cows and referable to identical or allied causes.   Therefore, the question has arisen in my mind, is there a most favorable period of gestation for dairy cattle.

It is a common occurrence for living calves to be born several days or even weeks before the end of the normal gestation period and it is of economic importance to know whether this abnormal physiological condition affects the productive ability of the animal.

In connection with this study a second problem was considered, that is, the relation between the age of the cow and the length of time she carries her fetus in utero.

REVIEW OF LITERATURE

Hammond (2) in summarizing the opinion of practical breeders of beef cattle, states there is no difference in the length of gesta-

tion of heifers and mature cows. He gives the average gestation for heifer calves as 38 weeks and for male calves as 41 weeks.

Wright (5) gives the average length of gestation for the cow as 280 days.

Wing (4) reports from the result of observations on one herd over a period of 10 years that the average gestation for 182 births was 280 days. The shortest period was 264 days and the longest 296 days. There were equal numbers for each day from 274 to 287 days. There was no difference in the average observed for males and females. Many cows showed well marked individual characteristics as to periods of gestation which may be several days longer or shorter than the average.

A summary of the breeding records of the Clemson Agricultural College herd (1), including Jerseys, Guernseys and Holsteins, shows an average length of gestation of 279.12 days for 194 females and 280.26 days for 192 males dropped in this herd. When the gestation period for animals at different ages, ranging from 2 to 14 years, was determined it was evident that no positive relation existed between the age of the cow and the length of her gestation period.

<center>SOURCE OF DATA</center>

Animals of the Jersey breed were chosen for study. Two groups were selected. One group of 406 cows selected from the 1926 and the 1927 supplements of the Register of Merit included only animals which had 365 day records made in AA class and which were milked three times per day or 1095 times during the year. The second group of 340 cows selected from the 1926, 1927, and 1928 supplements of the Register of Merit contained only animals with 365 day records made in AA class and milked twice per day or 730 times during the year.

Through the courtesy of Mr. L. W. Morley, Secretary of the American Jersey Cattle Club, the dates of service of the dams of these cows were secured. The dates on which these cows were dropped and also the dates on which their dams were dropped were secured from the volumes of the Jersey Herd Register.

In order to secure comparative values from records studied, all

TABLE 1

Correlation surface for butterfat production of Jersey cows milked three times per day and the length of time they were carried in utero by their dams

CLASS—COWS' YEARLY BUTTERFAT PRODUCTION	LENGTH OF GESTATION PERIOD IN DAYS														FREQUENCY
	253	256	259	262	265	268	271	274	277	280	283	286	289	292	
pounds															
400–449							1	2	1	1	3	1			9
450–499			1			1	1	2	6	4	6	1	1		23
500–549						1	3	4	4	8	3	1	1		25
550–599							1	7	10	18	12	2	2		52
600–649	1		1		1		3	8	16	22	16	7	2	1	78
650–699							4	10	11	24	11	3	1	1	65
700–749					1		5	5	12	17	10	8	2		60
750–799			1	1	2		3	1	11	14	7	2	1		43
800–849								3	5	5	6	2		1	22
850–899								4	5	3	2	3			17
900–949							1	2	1		1				5
950–999							1	1			2				4
1000–1049										1	1				2
1050–1099											1				1
Frequency..	1	0	1	2	2	5	23	49	82	117	81	30	10	3	406

TABLE 2

Correlation surface for butterfat production of Jersey cows milked twice per day and the length of time they were carried in utero by their dams

CLASS—COWS' YEARLY BUTTERFAT PRODUCTION	LENGTH OF GESTATION PERIOD IN DAYS													FREQUENCY
	256	259	262	265	268	271	274	277	280	283	286	289	292	
pounds														
400–449	1				1	1	4	2	15	7	5			36
450–499		1		2	1	3	13	17	29	21	2	3		92
500–549			1		1	6	5	13	22	13	3	2		66
550–599					3	4	11	15	11	14	3	2	1	64
600–649					1	2	3	11	12	9				38
650–699							3	5	11	4		1		24
700–749		1			1	1		2	2	2		1		10
750–799								1	3	1	2			7
800–849										1				1
850–899								1						1
900–949														0
950–999									1					1
1000–1049														
Frequency..	1	2	1	2	8	17	39	67	106	72	15	9	1	340

records were converted to a mature equivalent basis by multiplying the yearly fat record by the different age conversion factors as used by Turner (3).

The term fat record hereafter in this discussion will refer to a mature or mature equivalent record.   In case an animal had more than one Register of Merit record the highest mature equivalent record was used.
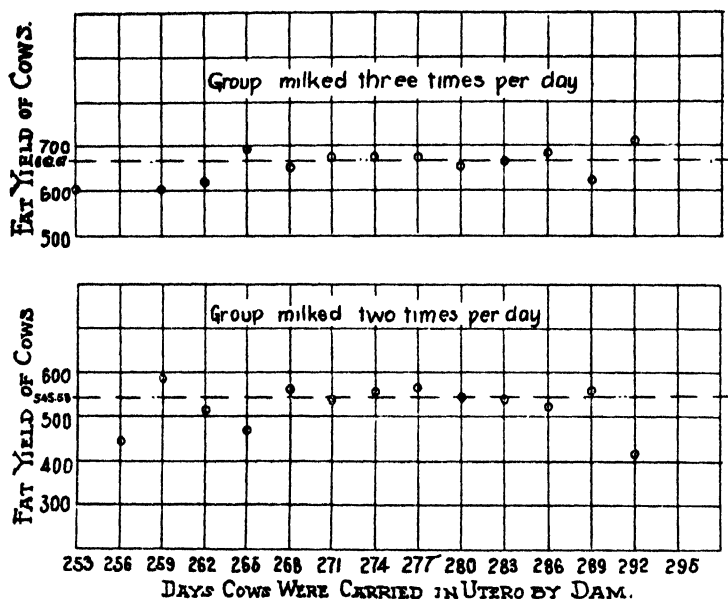
FIG. 1. AVERAGE BUTTERFAT PRODUCTION OF COWS CARRIED DIFFERENT LENGTHS OF TIME IN UTERO

PRESENTATION OF DATA AND DISCUSSION OF RESULTS

In the first study the mature equivalent butterfat records of the two groups of cows were grouped according to the length of time each cow was carried in dam as shown in the correlation surfaces (tables 1 and 2).

The coefficient of correlation for the data presented in table 1 is 0.0135 ±0.0335 and for that in table 2 is 0.0076 ±0.0366.

The arithmetical averages of production for the different length

of gestation groups were determined and are presented graphically in figure 1.

In each case the coefficient of correlation is smaller than the probable error. Also, when the averages are plotted it will be noted that each group falls very close to the mean for the entire lot.

TABLE 3

Correlation surface for age of Jersey cows and the length of their gestation periods

CLASS—AGE OF COWS	LENGTH OF GESTATION PERIOD IN DAYS														FRE-QUENCY
	253	256	259	262	265	268	271	274	277	280	283	286	289	292	
years															
1						3	2	1	7	8	7	1			29
2		1	1	1	2	4	5	13	29	30	23	3	2		114
3					2	4	22	24	47	24	12	5			140
4						6	20	15	19	16	5	2			83
5					1	5	5	14	28	15	7	4			79
6	1			1		3	6	8	13	27	14	6	2	1	82
7							2	3	9	19	15			1	49
8			1	1			2	2	11	12	10	4	2		45
9			1		2		2	2	6	12	8				33
10							2	1	8	6	4	1			22
11							2	1	6	4	2	1		2	18
12							1	1	3	1	3	2			11
13									3	1	1				5
14								1	1	2	2	1			7
15									1	1	3		1		6
16								1			1				2
17															0
18								1							1
Frequency	1	1	3	3	4	13	39	82	150	218	147	43	18	4	726

According to these data there is no indication that any particular length of fetal development has a significant effect on the butterfat production of the cow if this gestation period is between 253 and 293 days.

In the second part of this study the length of the gestation periods were grouped according to the age of the cows as indicated in the correlation surface (table 3).

The coefficient of correlation for the data presented in table 3 is 0.0551 ±0.0249.

The average lengths of gestation for the different age groups are presented graphically in figure 2.

The coefficient of correlation and the small degree of variation of the averages of the different age groups from the mean length of gestation for the entire group indicates that the length of gestation is not influenced by the age of the cow.
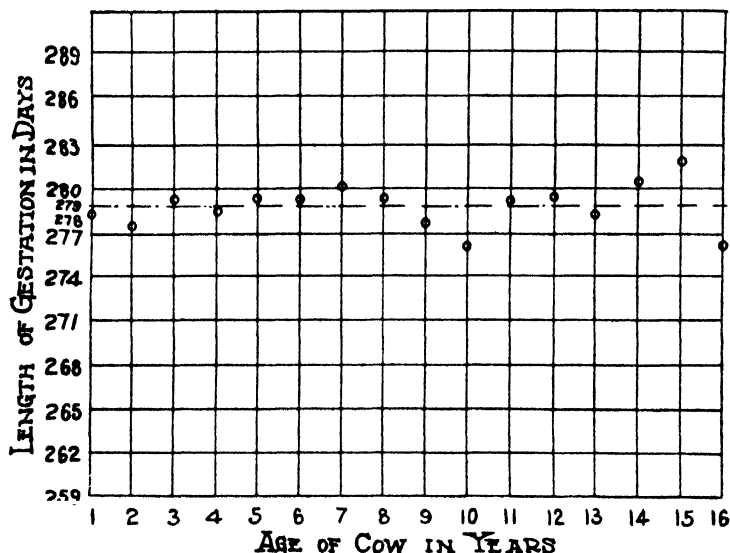
FIG. 2. AVERAGE LENGTH OF GESTATION OF JERSEY COWS AT DIFFERENT AGES

Attention is called particularly to the average length of gestation of all groups, including 726 animals, which is 278.9 days, a somewhat shorter period than is generally accepted as the normal gestation period for Jersey cows.

SUMMARY

1. These data indicate that the mature equivalent butterfat records of Jersey cows are not influenced by the length of time these cows are carried in utero by their dams.

2. The length of time these cows were carried in utero was not influenced by the age of their dams.

3. The average length of the gestation period for 726 Jersey cows was 278.9 days.

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SOME OBSERVATIONS ON PROCESSED CHEESE*

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Processed cheese is a comparatively recent product, but it has taken a very important place in the cheese industry, a high percentage of the total cheese output being marketed in this form. The advantages of this form of cheese have been very clearly stated by Davel and Retief (1).

In its manufacture there are various factors that affect the flavor, appearance and physical properties of the finished product. The only knowledge available on these factors is such information as has been accumulated in practical work. It is the object of this paper to give a report on these factors from a more scientific standpoint.

In the experimental work the following factors were studied:

1. The addition of the various so-called emulsifiers
2. The temperature treatment in processing
3. The moisture content
4. The reaction of the cheese
5. The age of the cheese used

To note the effect of these factors, observations were made on the behavior of the cheese in processing and on the following properties of the finished product:

1. The moisture content
2. The reaction, both pH and titratable acidity
3. The body of the cheese
4. Slicing properties
5. Color
6. Keeping quality
7. Appearance of the tinfoil

In addition to the above, there have also been some observations made on the processability of frozen cheese and the distribu-

tion of nitrogen between the water and 5 per cent salt soluble forms in the cheese before and after processing.

The kettle used, figure 1, was made in the University shops from plans drawn by us after the general design of kettles used commercially. When filled to capacity, it could handle about sixteen pounds of cheese, but for the experimental work it was decided to use eight pounds as this was enough to cover the bulb of the thermometer and could be easily handled. To empty the kettle the entire stirring device was raised without stopping its action and this in turn raised a plug in the bottom of the kettle that permitted the molten cheese to flow out.

In order to have a record of the temperature of the cheese in the kettle, a special thermometer was devised, the bulb of which formed part of the stirrers. This was connected by means of a fine copper tube to a glass capillary tube. The entire system revolved with the stirrers and the temperature was read on a stationary scale mounted in back of the capillary. The liquid used in this thermometer was xylene colored with Sudan III. With this device, it was possible to note the temperatures at which changes took place in the cheese during the heating.

As the body of the cheese is a criterion of its quality, it was thought advisable to have some means of determining the body in which the personal factor would be eliminated as far as possible. This was accomplished by means of the instrument shown in figure 2, which was designed especially for this purpose by the authors. The body of the cheese was measured by determining the force required to crush an inch cube to one-half of its original thickness. The force was applied by allowing mercury to flow into a beaker, placed as shown, and the stream of mercury was automatically shut off by an electrical device when the cheese had been crushed to 0.5 inch in thickness. The results are reported throughout in terms of the weight of the mercury and the beaker. To give the actual force applied, the figures must be multiplied by 5 since the mechanical advantage of the pulleys shown is 5. The crushing strength of cubes from the same sample of cheese differed appreciably due to the unavoidable variations
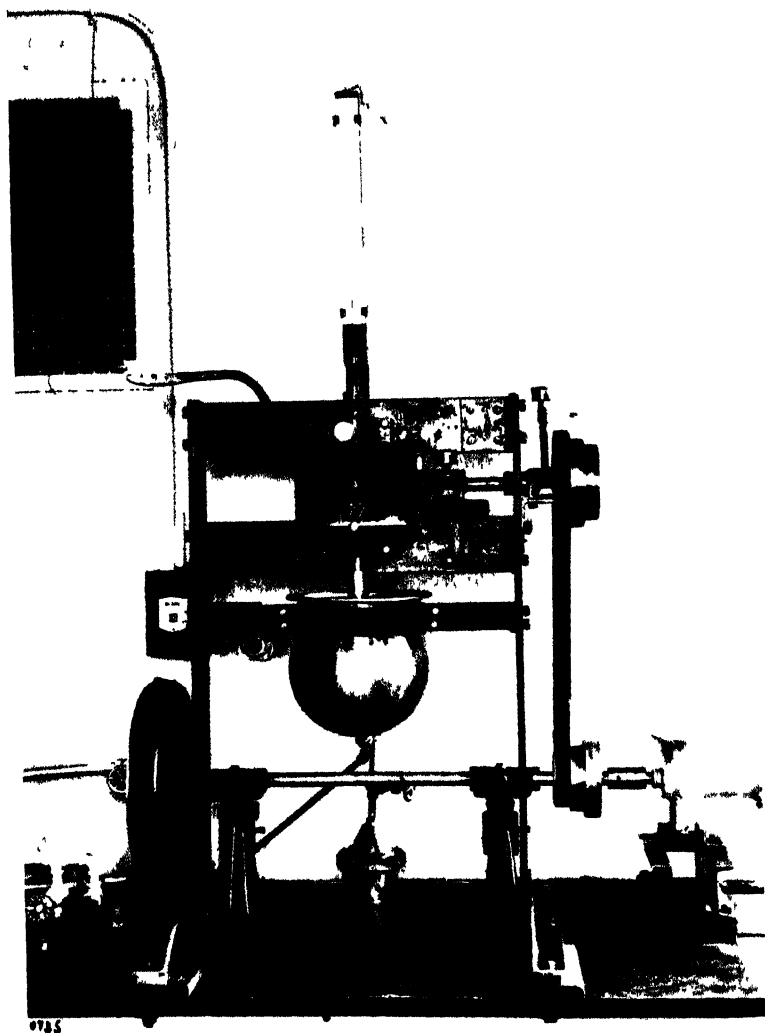
FIG. 1 EXPERIMENTAL CHEESE PROCESSING KETTLE

within the cheese. For this reason from 6 to 10 measurements were made on each sample and the results reported here are averages as too much space would be required to present all the data that have been collected.
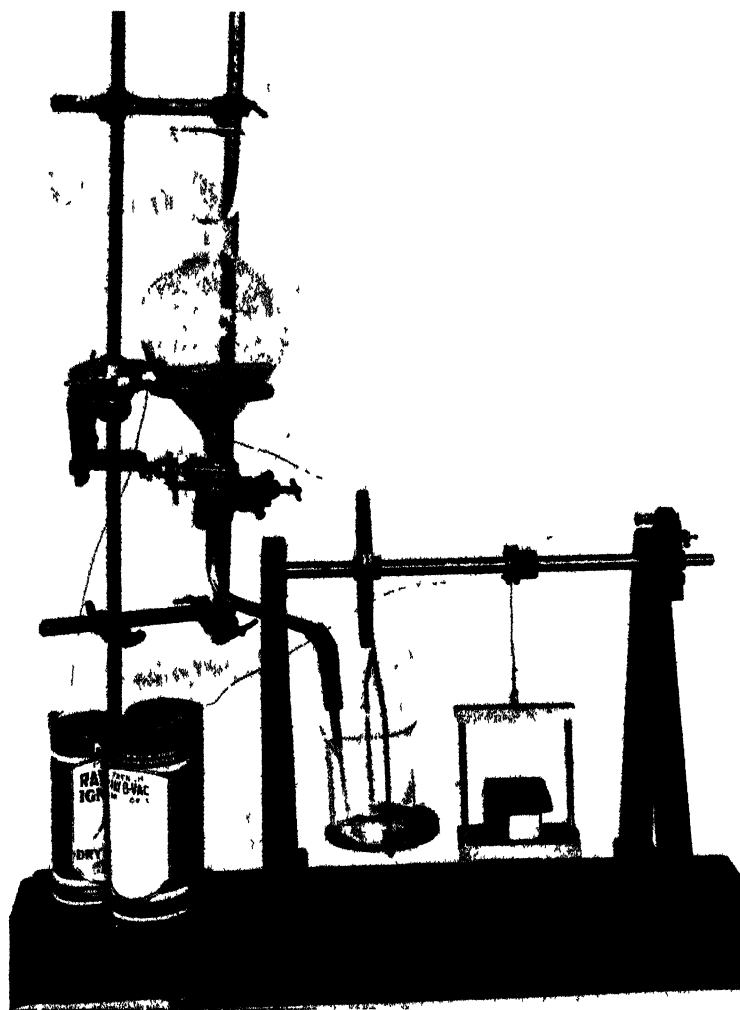
FIG. 2. DEVICE FOR MEASURING THE BODY OF CHEESE

EXPERIMENTAL

With this description of the apparatus used, let us now turn our attention to the salts commonly added to cheese, in addition to the common salt (NaCl) of which about one-half of one per

cent is used.   For want of a better term, these salts are commonly called emulsifiers.   Three have been used quite extensively either alone or in mixtures.   They are sodium citrate, di-sodium phosphate containing traces of the tri-sodium salt, and sodium potassium tartrate or Rochelle salts.   These salts are added for the purpose of preventing fat separation and controlling the body and texture of the cheese during and after processing.   The separation of the fat may be affected by the age of the cheese used, the reaction, moisture content, and the rate of stirring.   It is possible to select cheese in which these factors are combined in such a balance that no fat separation will occur at any time, and again the fat may separate as the heating is started and be reincorporated as the temperature approaches 130°F.   In such cases the use of these salts is unnecessary in the prevention of the fat separation, but even in these cases their addition may be decidedly bereficial on the body and texture of the finished product.

At present there are no tests that can be used to give an indication of the processability of cheese at the time of its selection except the use of a small experimental kettle.   As cheese is chosen on the basis of quality, especially flavor, and not with reference to its action during processing, the use of salts that have a controlling effect on fat separation and on body and texture is of importance to the commercial operator.

At the present time the use of Rochelle salts is on the decrease, due possibly to the fact that gritty, "sandy" particles are sometimes found in the cheese.   These crystals have been identified by us as calcium tartrate.   A series of experiments using Rochelle salts as the emulsifying agent failed to show any remarkable results in the processed cheese.   Besides the presence of calcium and tartrates in the cheese, there seem to be other undetermined factors involved in the formation of the gritty calcium tartrate crystals.

The curves shown in figure 3, give a comparison of the effect of di-sodium phosphate and sodium citrate, used separately and in mixtures, on the body of cheese.   It will be noted in each case that the citrate gives the firmer body and that the body decreases with the increase of phosphate in the mixture.   It is impossible to give

a very definite figure for the best body, but after a consideration of the results of tests made on experimental and commercial samples of cheese, it would seem that an inch cube of cheese requiring a crushing force of 800 to 1200 grams with a mechanical
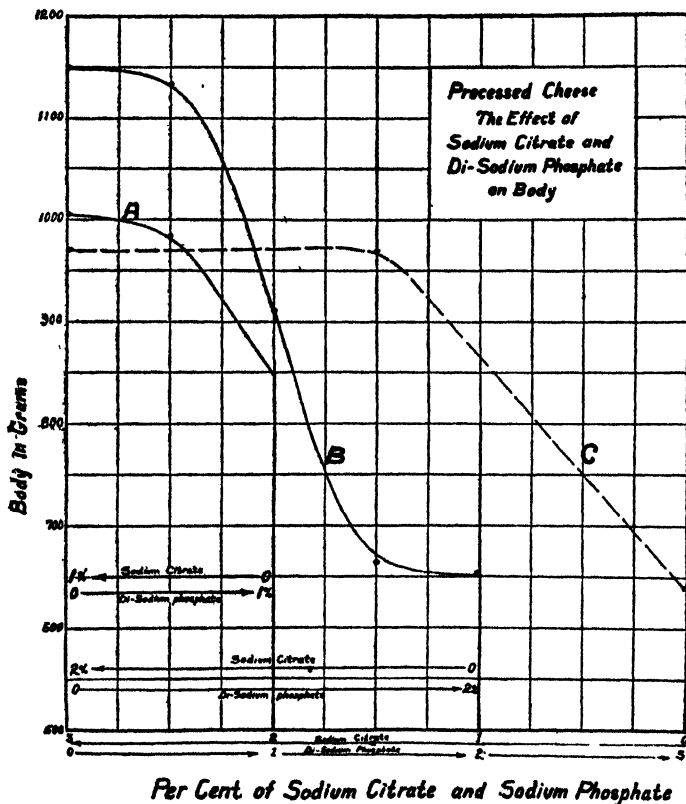
Fig. 3

The total amounts of the two emulsifiers, used alone and in combination, in graphs A, B, and C were always 1, 2, and 3 per cents, respectively.

advantage of 5, should be considered as having a desirable body. The citrated cheese in each case falls within these limits.

When the amount of di-sodium phosphate is increased beyond the limits shown in the curves, the body becomes more firm and then as the amount of the salt is increased, this is succeeded by a

weaker body accompanied with fat separation and grainy texture. With sodium citrate the addition of more of the salt did not cause any very marked changes in the body of the cheese and the use of 9.8 per cent sodium citrate did not cause fat separation. For commercial cheese-making the use of more than 2 per cent of these salts, especially the phosphate, should be avoided as it tends to give the cheese a bitter flavor when used in larger proportions, and the tin foil is darkened. Citrate on the other hand showed no evidence of darkening the tin foil except when very large amounts were used or when some alkaline salt such as sodium carbonate was added. When the reaction of the cheese, in terms of pH, was above 6.3, the tin foil would be discolored, and the darkening increased as the reaction approached neutrality. Unless it is stated otherwise, sodium citrate was used as the emulsifier in all the experimental cheese made.

Another interesting fact was brought out when cheese mites invaded the room in which the cheese was stored. They rapidly attacked the cheese in which citrate was used, whereas the cheese in adjacent boxes that was emulsified with phosphate was either untouched or only slightly eaten. The action of the cheese mites tended to give cheese a more alkaline reaction. While this action of the cheese mites may be taken as indicative of a preservative action of the phosphate, the manner in which the citrated cheese was consumed would seem to be evidence that the mites preferred it to the other.

A comparison of two forms of sodium citrate, namely, $2Na_3C_6H_5O_7 \cdot 11H_2O$ (U.S.P. VIII) and $Na_3C_6H_5O_7 \cdot 2H_2O$ (U.S.P. X) failed to show any differences in the action of the two salts.

A study of the temperature to be used in the processing of cheese was made from the standpoint of the degree that must be reached and also the length of time that the cheese may be held at the temperature. From the curve shown in figure 4, it is evident that there is little change in the body of the cheese when it is drawn from the kettle as soon as possible after temperatures of 140° to 150°F. had been reached. In cheese processed below 140°F. there was a formation of gas during storage indicating that these temperatures were insufficient to inhibit the growth of

bacteria. The odor of the cheese gave further evidence of putre-
factive changes. With the higher temperatures the body of the
cheese increased rapidly. In attaining these temperatures, there
is, of course, greater opportunity of evaporation and this in part
may account for the firmer body, but there are undoubtedly other
factors. With the higher temperatures, it was noted that the
color changed to a "salmon pink," the intensity increasing the

Processed Cheese
The Effect of
Processing Temperature
on Body
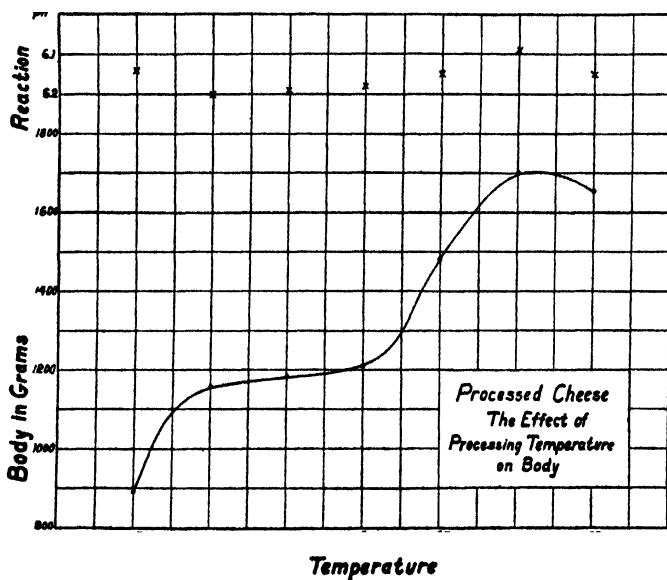
Temperature

FIG. 4

temperature. This change of color confirms the observations of
Davel and Retief (1).

When the cheese was held at 140° to 155°F. for 3 to 10 minutes
after reaching the desired temperature, the increase in body was
almost directly proportional to the holding time. There was
very little change in color at these temperatures, but holding for
any length of time at the higher point caused a distinct color
change.

In considering these temperatures it must be remembered that
the thermometer bulb was in the center of the mass of cheese and

was revolving with the stirrers at all times.   With the type of stirrer used and the speed at which they revolved, it was safe to assume that all the cheese was heated to the temperature recorded by the thermometer.

For all experimental work except when otherwise indicated the cheese was drawn from the kettle as soon as possible after the temperature had reached 148° to 150°F.
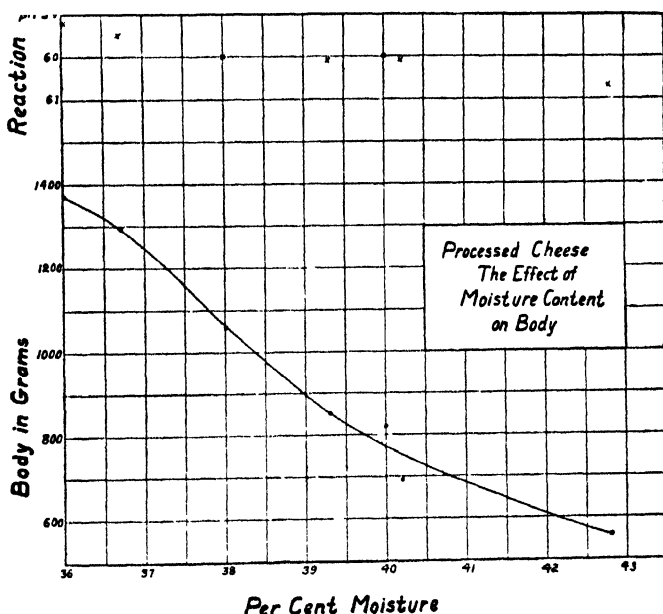
FIG. 5

The relation between the moisture content and the body of the cheese is shown by the curve in figure 5.   This indicates that as long as all other factors are kept relatively constant the body of the cheese will decrease with the increase in moisture.   It will be noticed that the figures previously given for a desirable body correspond with moisture contents of 38 to 40 per cent.   There seems to be a rather close relationship between the amount of water in a cheese and the way in which it can be cut with a commercial meat slicer that has been set to cut a section 0.02 inch in thickness.   If

the cheese contains from 37 to 40 per cent moisture, it will be cut quite easily and give a very uniform slice.   Below this the cheese is rather brittle and breaks as it is cut, while cheese containing excess water tends to stick to the revolving knife, and will not cut evenly except when the knife has been set to cut a thicker slice.

Studies on the loss of moisture during processing indicate that there are a number of factors that must be considered.   If the kettle is of the wide and rather flat type, it will be necessary to use more water than if the kettle has more nearly vertical sides. The amount of cheese in the kettle is of importance also as there is a greater loss proportionally with a small amount than if the kettle is filled nearly to capacity.   The actual loss may vary from practically none to as high as 3 per cent, and if the time necessary to reach the desired temperature is long, the loss may go as high as 5 per cent.   In commercial work the man operating the kettle will, after some practice, be able to estimate very closely the amount of water that must be used to give cheese with the required moisture content.

In the work described in this paper, the water of crystallization of the emulsifiers was not taken into consideration as in most cases it was less than the amount necessary to bring the water content of the salts added up to the 40 per cent limit.   When other factors were being studied, the amount of water added was sufficient to give the finished product 38 to 39 per cent moisture.   In the experimental cheese where rather large amounts of phosphate were used (approximately 50 per cent water) the moisture content of the finished product was somewhat higher than 39 per cent).

It is possible to have wide variations in the body of the cheese and still keep the amount of moisture present nearly constant.   A most striking example of this will be pointed out on another curve in which two adjacent points have a difference of 2000 grams in body and only 0.2 per cent difference in moisture with the cheese having the higher moisture also having the higher body.   If an excess of water is added during the processing, the body of the cheese is weakened, there is a tendency for the fat to separate in spite of emulsifiers, and the finished product may be best described as coarse and grainy.

When the relation between the body and the reaction is considered, the very interesting curve shown in figure 6 is obtained.
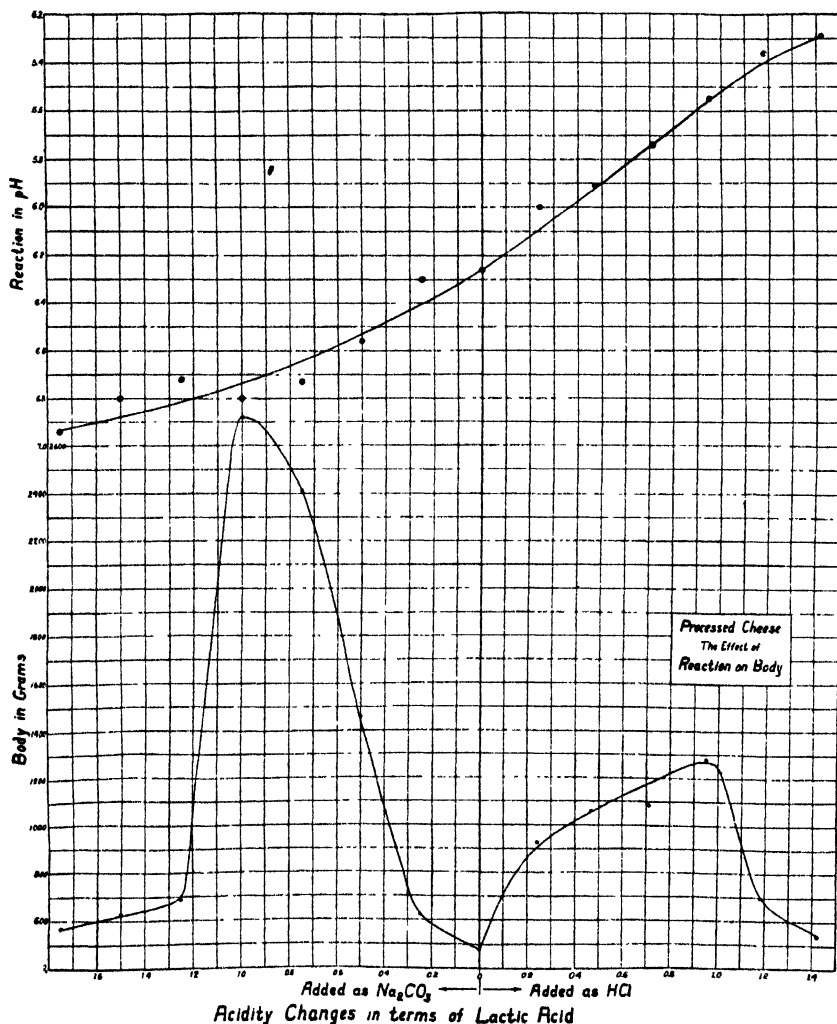
FIG. 6

In this series of experiments the amount of emulsifier used was kept constant and the other reagents added in the amounts indicated. The increase in the body with the addition of alkali is

very remarkable and the sudden break beyond 1 per cent is rather unexpected. It is at this break that we find the previously mentioned difference in body with the moisture content remaining the same. If one plots the reaction in terms of pH against the body, a curve is obtained that is practically identical with the one shown. As was to be expected from the work on the emulsifiers and the use of phosphate, the tin foil of the more alkaline cheese was very dark and this color was imparted to the cheese so that there was a discolored layer on the outside of the cheese that was in some cases nearly half an inch deep. This cheese was also gassy while the cheese at the more acid reaction was not, which would indicate that the reaction of the cheese may influence the pasteurizing temperature to be used. The acid side of the curve shows an increase in body, that is not as pronounced as the other and the break is less abrupt. It is not necessary to use a mineral acid to get a curve of the nature shown as a similar one was obtained when a part of the sodium citrate used was replaced by an equivalent amount of citric acid.

The reaction curve shown in the same figure indicates a fairly uniform change with each addition of the reagents and is more uniform on the acid side than on the alkaline. This is no doubt due to the combination of the reagents with the casein and fat of the cheese and is best illustrated by the texture of the cheese. With the more acid cheese the body was coarse and grainy giving a very rough surface when cut showing fine particles, there was also a greasy film around the cheese. With the alkaline cheese there was less graininess and a tendency towards soapiness. The layer of fat on the outside was deeper and the taste of the cheese was very disagreeable being very bitter as compared with the sour taste of the more acid cheese. When citric acid was used in small amounts (0.1 to 0.2 per cent) the taste of the product was rather pleasant giving the impression that the original cheese was somewhat older than was actually the case, but if the amount of acid was increased, the sour flavor was more pronounced making the cheese rather unpleasant to the taste.

It is impossible to show any very definite relationship between the titratable acidity of the cheese as expressed in per cent of lac-

tic acid and the body. The titratable acidity in connection with
the reaction in terms of pH may be used to give an idea of the
kind of cheese and the emulsifier that was added when examining
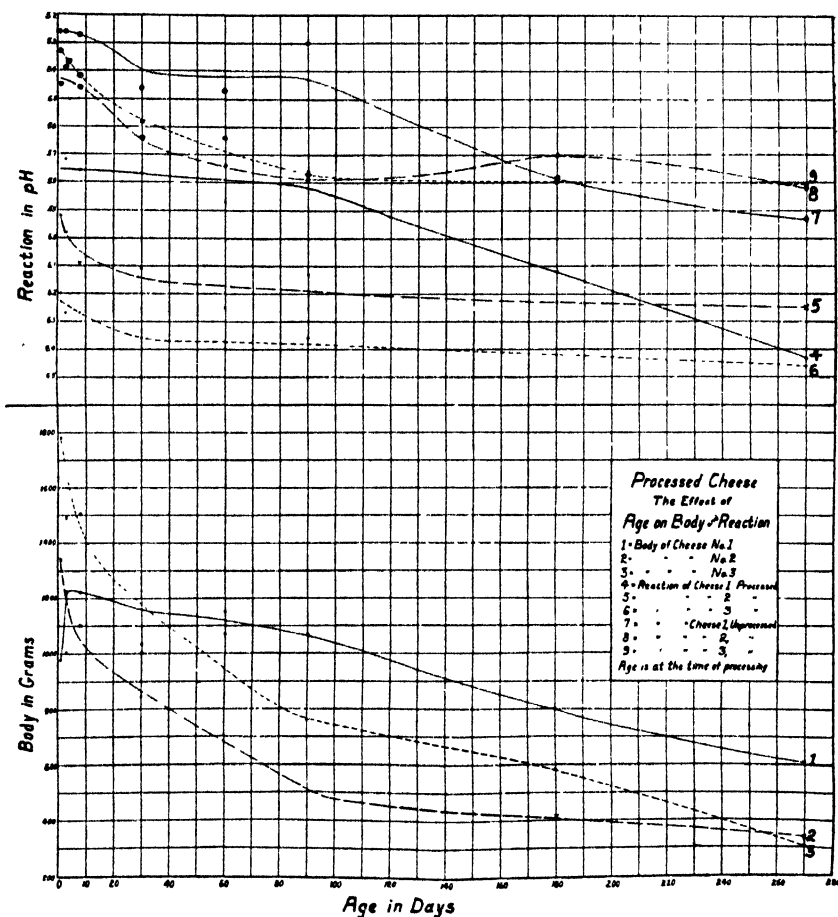
Fig. 7

cheese of unknown processing history. A high value for the
titratable acidity coupled with a low pH (5.8 or lower) would
indicate that either a very acid cheese had been used or that acid
had been added during the processing. If, however, the titrat-

able acidity is high and the pH high (6.4 or higher) this could be considered as evidence of the use of an excessive amount of emulsifier especially phosphate. A low titratable acidity with a high pH would be indicative of the addition of free alkali at some time during the making of the cheese.

The reaction of typical processed cheese in terms of pH may vary from 5.8 to 6.3. The titratable acidity may range from 1.1 to 1.8 per cent calculated as lactic acid. With the blending of the cheese as it is done commercially, the reactions of the resultant product stay well within these limits.

In order to study the effect of age upon the processability of cheese, experiments were conducted with three series of cheese. The cheese was made up as Young Americas (each containing approximately ten pounds) so that a single cheese would furnish material enough for an experimental batch in processing. One cheese from each series was processed at the ages indicated on the curves shown in figure 7. Two had sodium citrate used as the emulsifier while the other, cheese 3, had di-sodium phosphate. The curves for the body show a very uniform decrease with the exception of cheese 1 for the first few days. Cheese 3 containing the phosphate had a much stronger body at first but the decrease with age was more rapid and after 9 months we find that the body is much poorer than the others. The curves for the reaction before and after processing show that there is a change of approximately 0.4 of a pH unit towards the alkaline side when sodium citrate is used, while di-sodium phosphate shows an average difference of about 0.6. Cheeses 2 and 3 were made on successive days and the parallelism between the reaction curves after processing is very striking.

With the very young cheese it was impossible to process it without the separation of fat even with the use of emulsifiers and the fat was not re-incorporated as the temperature increased. After the cheese was 8 days old, this difficulty was not encountered again with any of these series. With cheese more than a year old, this difficulty may occur again, but it can be overcome to a large extent by increasing the amount of the emulsifier. The cheese at the age of 9 months tended toward a very grainy texture

and the use of phosphate seemed to make this defect more pronounced. With the young cheese, the body was very firm and the cheese as it came from the kettle did not flow together but formed rather distinct layers. Considerable air was held in such cheese in pockets of various sizes making it rather difficult to secure inch cubes that were free from these imperfections. These pockets often contained free butterfat that had been carried along as the cheese was run out of the kettle. The flavor of this young cheese was the same as the unprocessed green curd and the texture was rather rubbery.

In the commercial manufacture of processed cheese, many of the difficulties that have been mentioned here as due to the age and reaction of the cheese used may be overcome by blending. This consists of the selection of cheese of such ages and degree of ripeness that the product will be free from all the defects that have been mentioned.

This is usually accomplished by mixing two or three parts of relatively young cheese, about two months old, with one part of older cheese, depending upon the flavor that is desired in the product. If the cheese has been properly chosen and the average age is about four to seven months, it is reasonable to expect a very satisfactory product.

In connection with a study on the freezing point of cheese (2), a series of experiments were conducted in order to determine whether freezing affected the processability of the cheese in any way. There were 6 samples of cheese of different ages and in various stages of curing. For each cheese frozen, there was a similar one that had been kept under ordinary storage conditions. Part of each cheese was processed within a week after it had been removed from the freezing temperature and the remainder was processed 6 weeks later after storage at 40° to 50°F. It was impossible to show any marked differences between the frozen and the unfrozen cheese during or after processing. With the former there appeared to be a slight fat separation with the younger samples as the heating started, but this was all re-incorporated before the cheese was drawn from the kettle. The results with

these cheeses confirmed those obtained previously with cheeses of similar age and reaction.

In connection with the work on the effects of age on the processability of cheese, some experiments were conducted on the distribution of the nitrogen between water and 5 per cent salt (NaCl) soluble. The results of these investigations are shown in table 1. The findings of Van Slyke and his co-workers (3) have been confirmed regarding the changes in the distribution of the nitrogen as the cheese ages. With the very young cheese, the salt soluble fraction was very much greater than the water soluble,

TABLE 1

The effect of age and processing on the nitrogen distribution in cheese

AGE	UNPROCESSED CHEESE						PROCESSED CHEESE					
	H₂O	Total nitrogen	Water soluble		Salt soluble		H₂O	Total nitrogen	Water soluble		Salt soluble	
			Nitrogen	Per cent of total N	Nitrogen	Per cent of total N			Nitrogen	Per cent of total N	Nitrogen	Per cent of total N
months	*per cent*	*per cent*	*per cent*		*per cent*		*per cent*	*per cent*	*per cent*		*per cent*	
1	36.51	4.00	0.77	19.25	1.24	31.00	36.8	3.67	3.15	85.83		
1	35.04	3.91	0.88	22.51	1.94	49.61	39.2	3.53	1.75	49.57	0.13	3.68
3	37.38	3.70	1.78	48.11	0.98	26.49	37.1	3.38	2.62	77.51	0.12	3.55
3	37.42	3.81	1.66	43.57	0.58	15.22	36.4	3.65	2.46	67.40	1.11	30 41
6	36.71	3.98	1.76	44.22	0.28	7.03	38.7	3.77	2.12	56.23	0.22	5.83
6	36.70	3.92	1.78	45.41	0.22	5.61	39.5	3.60	2.73	75.83		
9	36.01	3.90	1.79	45.12	0.58	14.87	38.5	3.48	1.81	52.01	0.19	5.46
9	36.25	4.03	1.71	42.43	0.44	10.92	38.1	3.68	2.40	65.22	0.22	5.97
9	34.49	4.18	2.05	49.04	0.21	5.10	37.6	3.57	2.26	63.31	0.09	2.52

but as the age increased the water soluble portion increased, and the salt soluble decreased quite rapidly. Besides the work on the unprocessed cheese, determinations were made on the cheese as soon as possible after processing and the results are shown in the second part of the table. The results were very interesting in respect to the very significant increase in the amount of water soluble nitrogen that was found after processing. In every case there was a decided increase although the actual amount showed a rather wide variation. The average increase calculated as the per cent of total nitrogen is approximately 26 per cent.

SUMMARY

From the results obtained in this experimental work, there are a number of conclusions that may be drawn:

1. The use of the so-called emulsifiers is beneficial and the results obtained with sodium citrate indicate that it is superior to di-sodium phosphate. The use of more than 3 per cent of these salts should be avoided not only to remain within the legal limits but also for the effect on the finished product.

2. Temperature regulation is essential in order to insure the proper pasteurization of the cheese and also to prevent the color from changing to "salmon pink." If the cheese is to be held in the kettle for any length of time, it should be at the lower pasteurization temperature and water should be added to make up for the losses due to evaporation.

3. Close observance of the standards set for the moisture content of processed cheese are essential not only for economic reasons, but also to insure having cheese of as uniform body as possible.

4. Good results were obtained with cheese ranging in reaction from pH 5.8 to 6.2. This would indicate that this factor can be adequately controlled by exercising discrimination in blending.

5. Very young cheese showed excessive fat separation in processing, and a rubbery texture in the finished product. Very old cheese also processed unsatisfactorily producing a product with a weak body and grainy texture. Good results were obtained when the blending was such that the average age of the cheese was from 4 to 7 months.

6. A comparison of the reaction in terms of pH and the titratable acidity furnishes a means of getting some ideas as to the kind of cheese that was processed and the treatment that it received.

7. Cheese that can be cut in slices 0.02 inch thick and requires 800 to 1200 grams with a mechanical advantage of 5 to crush an inch cube to one-half of its original thickness may be considered as having a very desirable body.

8. The keeping quality of processed cheese is dependent upon

the heat treatment, the reaction and to some extent upon the temperature of the store-room.

9. Discoloration of the tin foil may be due to the use of phosphate as the emulsifier or to the use of alkali giving a reaction above pH. 6.3.

10. Frozen cheese can be processed satisfactorily.

11. Processing increases the water soluble form of nitrogen in the cheese.

In conclusion the authors wish to express their thanks to the Chas. Pfizer and Company, Inc., who have sponsored the fellowship under which this work has been done.

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STUDIES ON OXIDATION-REDUCTION IN MILK

THE METHYLENE BLUE REDUCTION TEST*

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In the first two papers of this series some theoretical aspects of oxidation-reduction phenomena in milk were discussed. The present paper deals with some of the more practical considerations of the methylene blue reduction test. The reader is referred to the previous articles (Thornton and Hastings, 1929) for a more complete discussion of the mechanism of reduction than space permits in this paper.

METHODS

The water bath employed was electrically heated and thermostatically regulated. The temperature used was 37.5°C. ±1°, unless otherwise stated. The temperature in different parts of the bath did not vary more than one-half degree.

Except where noted 10 cc. portions of milk in test-tubes were used. The methylene blue tablets manufactured by the National Anilin and Chemical Company were used. Unless reported to the contrary one tablet was dissolved in 200 cc. of distilled water and this solution was sterilized by steaming. One cubic centimeter of this solution was added to 10 cc. of milk, giving an approximate concentration of 1 part of dye to 200,000 parts of milk (Standard Methods of Milk Analysis, 1928). Dye concentrations as reported in this paper are, therefore, only approximate.

The end-point of reduction was considered reached when the milk appeared white on closing the tube and inverting it once to mix the contents thereof. All reduction times are reported in hours and minutes. Thus, 13:07 means 13 hours and 7 minutes.

The heading "Time" in the tables means reduction time. Care was taken to have all equipment sterile except in those cases calling for other technique.

In the plate count work Standard Methods of Milk Analysis (1923) were followed with the exception that 1 per cent of glucose was added to the medium and the counting was done without the aid of a reading glass. Three plates were poured from each dilution and not less than 2 dilutions were plated per sample. Incubation periods were for 2 days at 37.5°C.

THE MECHANISM OF REDUCTION

Since there is no oxygen in the methylene blue molecule, the reduction of this dye involves a transference of hydrogen. The source of this hydrogen is still uncertain. It seems probably that many compounds act as hydrogen donators. These have been called metabolites. To these belong such substances as the sulfhydryl compounds, citrates, succinates, aldehydes, and others. Lactose must also receive consideration as a factor in reduction in milk.

Methylene blue may act as an acceptor for the hydrogen and undergo reduction. Molecular oxygen dissolved in the milk may also be reduced by the hydrogen, according to the theory of Wieland, first forming H_2O_2 which is then reduced to H_2O. Coincident with the oxidative and reductive changes in milk, potential changes are also observed. The potential values of fresh milk fall within a rather narrow range. As dissolved molecular oxygen disappears the potentials of the milk become more negative. At the more positive values the oxygen seems to have a greater affinity for the hydrogen than does methylene blue. When a certain potential is reached, the situation appears to be reversed and the hydrogen reduces the dye (Clark, Cohen, and Gibbs, 1925). The removal of oxygen by mechanical, chemical, or biological means causes a fall of potential in the milk. Barthel (1917), and Thornton and Hastings (1929) washed the molecular oxygen out of fresh raw, and sterile milk with inert gases and observed reduction of methylene blue. Barthel (1925) reports reduction of this dye in a sterile mixture of lactose, peptone, kaolin,

milk salts, and sodium citrate. Dubos (1929), and Thornton and Hastings (1929) studied the reduction of dyes in sterile bacteriological media. Coulter (1928) reported the oxidation-reduction potentials of sterile nutrient bouillon. Burri and Kürsteiner (1912) showed that reduction of methylene blue takes place in fresh milk in which bacterial action is inhibited by antiseptics. They thought that dissolved oxygen plays an important rôle in this test. Thornton and Hastings present evidence that milk itself consumes oxygen, a characteristic probably common to all organic complexes.

Barthel (1917) believed that the disappearance of methylene blue in milk takes place in two stages, viz.:

1. The removal of the dissolved oxygen by the growing bacteria
2. The reduction of the dye by constituents of the milk

There are today considerable data in support of this theory. It is probable that minute but inconsequential amounts of oxygen are fixed by constituents of the milk during the operation of the test. Some investigators believe that reduction of the dye takes place within, or at the surface of, the bacterial cell. We are of the opinion that at least the major portion of the dye is reduced independently of the cell. All of our work tends to confirm the theory of Barthel. Therefore, the reduction time of methylene blue in raw or pasteurized milk may be looked upon as a criterion of the oxygen-consuming power of the bacteria in the milk.

A CONSIDERATION OF OXYGEN RELATIONSHIPS

The importance of the rôle of oxygen in the methylene blue reduction test has been recognized to the extent that some have mentioned the possibility of inaccuracy due to variations in the oxygen content of different milks. It has been advised that the milks be given a preliminary shaking to bring them into equilibrium with the atmosphere, and then be protected from the diffusion of atmospheric oxygen during the test by covering with vaseline or paraffin. We have shown in our previous papers that the rate of diffusion of atmospheric oxygen into whole milk is so slow as to have no measurable effect upon reduction times if the tests

are carried out in the usual culture tubes. The protective effect of the cream layer and of the aerobic flora, which is probably universally present, is sufficient to account for these results. In only one case did we find a difference between the reduction times' of protected and 'unprotected tubes of skim milks.

Marshall (1902) reported the oxygen content of the gas from six samples of milk, obtained by the ordinary methods of milking, as being:

Milk number......	1	2	3	4	5	6
Per cent oxygen..	13.79	12.66	14.17	11.89	10.96	15.57

The total amount of gas in these milks was fairly constant. Different methods of handling the milk subsequent to milking, such as aeration over glass, tin, copper, glass wool, etc., caused an appreciable increase in the oxygen content of the gas. There is likely to be, as these figures show, some variation in the oxygen content of different milks as received at the milk plant. The data which we are presenting show that inaccuracies in the test due to this variability are probably small.

Lojander (1925) claimed that shaking the milk 30 times results in a lengthening of reduction times. Other workers, as Hastings, Davenport, and Wright (1922) found that a preliminary shaking has practically no influence upon reduction times. We have not observed that preliminary shaking makes any appreciable difference in results.

An experiment in which the milk was shaken for 15 minutes is reported in table 4. In another experiment oxygen was bubbled through the milk for 5 minutes prior to the addition of the methylene blue. In neither experiment was the reduction time appreciably affected. These results showed that the original milk was in approximate equilibrium with the air. We have observed nothing leading us to believe that differences in the oxygen content of milks produced in the ordinary way will introduce serious inaccuracies into the test. It can be assumed, therefore, that the reduction times of methylene blue in milk, as observed in the standard operation of this test, are influenced almost entirely by the rate of oxygen uptake by the milk bacteria.

The rates of oxygen uptake by different species of bacteria are not well known (Callow, 1924; Burnett, 1927; and Soule, 1928). It is probable that the rates vary with different species, and with different conditions, sufficiently to introduce an important factor of inaccuracy into the test. This, we believe, is one of two important sources of inaccuracy, the second being the removal of bacteria from the body of the milk by the rising butterfat. A knowledge of the rates of oxygen uptake by bacteria would be of doubtful value, as far as this test is concerned, since the flora of the milks subjected to the test in practice is an unknown factor. This situation is relieved, somewhat, by the tendency of the lactic acid bacteria to predominate in most of the milks to which the test is applicable.

Barthel (1908) and Orla-Jensen (1912) reported the lactic bacteria to be slow reducers, while Fred (1912), Rahn (1920) and Hastings, Davenport, and Wright (1922) classed them among the strongly reducing organisms. The latter authors pointed out the reason for this divergency of opinion. Barthel and Orla-Jensen added methylene blue to cultures of the bacteria in which growth had already taken place. In the experiments of the other workers the dye was added before growth had been retarded. As we have already seen, the reduction of methylene blue in milk is the result of bacterial activity. Under the first condition the oxygen-consuming capacity of dormant bacteria was in question; under the second the capacity of growing organisms was in question. The rate of oxygen uptake by reproducing cells is several fold that of dormant cells.

The bacteriologist frequently uses plant and animal tissues to produce anaerobic conditions. The oxygen-consuming and the reducing properties of living plant and animal tissue are recognized. It is logical, therefore, to suppose that leucocytes have this power and that they are a factor in the reduction of methylene blue in raw milk. Cases have been reported in which the reduction times of certain samples of milk have been disproportionate to the bacterial content of the milk as revealed by the plate or by direct microscopic counts. Skar (1913) studied this question and established the reducing power of cells from the

lymph glands and of leucocytes from centrifuged milk. A mathematical relationship between leucocytes and reduction times has not been established as has the relationship between bacteria and reduction times. The effect of age and temperature upon the reducing activities of these body cells is not known. It is improbable that the leucocytes play a major rôle in the reduction test except, perhaps, in long-time reducing or abnormal milks.

THE EFFECT OF VARIATION OF pH

Mueller (1906) showed that the neutralization of considerable produced-acid in milk does not change its reduction time. This

TABLE 1

Plate counts in millions taken at the moment of reduction

¼ HOUR REDUCTION TIME	½-1 HOUR REDUCTION TIME	1-2 HOURS REDUCTION TIME	2-3 HOURS REDUCTION TIME	3-4 HOURS REDUCTION TIME	4-5 HOURS REDUCTION TIME	5-6 HOURS REDUCTION TIME
38	35	16	3.5	44	25	35
19	35	12	11	13	10	45
80	31	8	23	20	32	4.5
32		17	16	5	19	5.5
		26	23	20		
		12	22	29		
		9.5		20		

has been confirmed by Orla-Jensen. In their series of papers on oxidation-reduction, Clark and his coworkers showed that at 30°C. the potential becomes more negative by 0.06 volt for each increase in pH equivalent to unity. The figures presented in our first papers of this series show that the change of pH during the operation of the test is slight. It is seen that any variations in pH met with in milks to which this test is applicable are not sufficient to have any measurable visual effect upon reduction times. This is precisely what one would expect, for it is well known that the rate of growth of the lactic bacteria is not checked until the milk has developed sufficient acid to cause curdling at ordinary temperatures. Hastings and Evans (1913) reported that the number of bacteria must reach approximately 100 million be-

fore any appreciable change is noted in the reaction of the milk as measured by titration.

The plate counts of 39 samples of milk, made at the moment of decolorization, are given in table 1. One sample, giving a plate count of 80 million, was on the verge of souring and reduced methylene blue in less than 5 minutes. With this exception the counts varied from 3.5 million to 45 million with an average of 22 million. Because of the wide variations in the plate count these differences are probably more apparent than real. Nevertheless, wide variations are likely to occur. That the differences between the numbers present in the long and the short time reducing milks are not constant or striking may be significant. This may indicate that resting cells have little effect upon reduction times in the usual operation of the test. These results show conclusively that the bacterial content at the moment of reduction, while high, is not sufficiently so to cause any great change in the reaction of the milk.

THE EFFECT OF TEMPERATURE

The relationship of temperature has been studied extensively by Rahn (1920) and by Hastings, Davenport, and Wright (1922). The ordinary water-bath is easily regulated, even when an alcohol lamp is used as the source of heat, so that temperature fluctuations need not be greater than 1 or 2 degrees. The work to which reference is made above points to the conclusion that inaccuracies in this test due to such small fluctuations in temperature would be comparatively small.

THE SWEEPING EFFECT OF THE RISING BUTTERFAT

When working with this test, one soon observes the irregular and uneven disappearance of the blue color from certain samples of milk. Sometimes the major portion of the milk will turn white leaving a blue band at the top, bottom, or in the middle. In a few cases white streaks or patches appear. Difficulty is experienced in obtaining a definite end-point. Vitoux (1920) recommends disregarding the upper quarter in reading the test. Another method involves closing the tube with the finger, inverting

once, thus mixing any unreduced dye persisting in any part of the tube with the entire mass of milk. If the milk appears white the end-point is considered to have been reached. The slight amount of oxygen incorporated in the milk by the inversion of the tube has no significant effect on the reduction time, as more violent agitation may have. It is frequently difficult to tell the exact end-point even in cases in which the disappearance of the dye is uniform. Much depends upon the sensitivity of the eyes of the observer to blue light.

Many workers have reported the reduction times to 3 minutes. Except in very rapidly reducing milks we have found it difficult to read the end-point, in many cases, to within 5 and even 10 and 15 minutes. In very slowly reducing milks this doubtful period is extended greatly. In one sample the end-point was taken as 25 hours, but it was simply guess work after 23 hours. Experience soon enables one to judge the end-point by the above method with a minimum of error. No pretense is made in this report that reduction times are correct to within 5 minutes except in the case of rapidly reducing milks. In the majority of cases times are reported to the nearest 15 minutes.

In attempting a determination of the accuracy of this test wide variations in the reduction times of duplicate tubes were noted. It was observed that these wide discrepancies appeared in the exceptionally good milks. Smaller and less serious variations were noted in some of the more mediocre milks. We have endeavored to find the cause for this and the results here reported are representative of a large number of such experiments. In one experiment 36 duplicate tubes were prepared in the standard manner. Of another sample of milk only 5 tubes were used. The results of the experiments are given in table 2.

These variations, we believe, are due to the uneven sweeping of the bacteria out of the milk by the rising butterfat. Skar (1913), observing this same variation, attributed it to the bacteria and the leucocytes being carried out of the milk by the butterfat and to a settling of some of the cells to the bottom of the tube. Not a great deal of stress has been laid on the importance of this source of inaccuracy in the reduction test.

Anderson (1909) found that gravity cream contained on the average approximately 15 times as many bacteria as the skim milk. Schmidt (1925) reported the lactic acid bacteria 85 times more numerous in the top than in the lower layers of milk. Leete (1926) gave the following figures as the average of 25 samples:

	Number of bacteria per cubic centimeter
Whole milk	135,880
Gravity cream	283,680
Skim milk	33,556

TABLE 2

Reduction time variations in duplicate tubes of two milks

SAMPLE 1		SAMPLE 2	
Number of tubes	Time	Number of tubes	Time
3	9:30	1	13:15
2	9:45	1	13:30
2	10:00	1	16:00
3	10:15	1	21:45
12	10:30	1	25:00
1	10:45		
2	11:00	5	Ave. 17:54
8	11:15		
1	11:30	Variation 11:45	
1	12:00		
1	12:30		
36	Ave. 10:48		
Variation 3:00			

Breed (1926) in working on the effect of gravity creaming on the body cell content of the cream and skim milk reported a tremendous sweeping of cells into the cream layer. In commenting on his results Breed writes,

These examinations show that when cream is allowed to rise by gravity the greater portion of the cells rise with the cream. The reason for this is simple. Many of the cells contain fat drops and therefore naturally tend to rise with the cream. Others of the cells adhere to fat drops and these are buoyed up with the fat drops as they rise. These

observations show that gravity cream may be invariably expected to contain large numbers of cells as the cells concentrate in the cream when it is formed in this way. . . . . Skim milks invariably contain few cells.

A simple experiment to prove this is to allow some tubes of good milk to sour. If half of these tubes are shaken frequently, while half are left undisturbed, the former will coagulate first.

If the above mentioned variations in the reduction times of duplicate samples of better class milks are due to the rising of the bacteria with the butterfat, then these variations should be lessened or even entirely wiped out if creaming is prevented. We have used different methods to accomplish this. The following

TABLE 3

Effect of shaking on reduction time

UNSHAKEN		SHAKEN	
Number of tubes	Time	Number of tubes	Time
1	10:15	1	6:50
1	10:50	1	6:50
1	11:05	1	7:05
1	11:10	1	7:05
1	12:05	1	7:05

are representative of a large number of experiments which have been made. Five duplicate tubes were incubated at rest and 5 were shaken every 15 minutes during the entire course of the test. The results are presented in table 3.

If the difference between the shaken and unshaken tubes is due to keeping the bacteria mixed with the milk, then it should make little difference whether the shaking is violent or gentle, frequent or infrequent (within certain limits). The following results are indicative of this. In the case of a milk showing a reduction time of 8 hours, 5 tubes shaken gently (2 or 3 times) every half hour reduced in 6:30, 5 tubes shaken violently (25 times) each half hour reduced in the same time. Similar results were obtained in a second trial with another milk.

It frequently happens with poor milks and occasionally with middle class milks that shaking increases the reduction.   With these milks quarter-hour and half-hour shakings give different results.   This is due to the incorporation of oxygen just at the time of decolorization, at which time the oxygen content of the milk is very materially lowered.

TABLE 4

The effect of preliminary shaking with glass beads on reduction time

NOT SHAKEN WITH GLASS BEADS		SHAKEN WITH GLASS BEADS	
Unshaken in bath	Shaken in bath	Unshaken in bath	Shaken in bath
time	*time*	*time*	*time*
10:15	6:50	9:05	6:35
10:50	6:50	9:25	6:35
11:05	7:05	10:15	6:35
11:10	7:05	10:15	6:35
12:05	7:05	10:20	6:35

TABLE 5

Reduction times when the milk was coagulated by rennet

SAMPLE NUMBER	UNSHAKEN CONTROLS	SHAKEN CONTROLS	PLUS RENNET
1	0:15	0:15	0:15
2	4:00–4:10	3:30	3:00
3	1:45–2:05	1:15–1:20	0:50
4	5:15	4:15	4:00
5	3:45	2:30	2:00
6	1:30	1:30	1:05
7	4:45	3:45	3:00

In order to find if the shaking was breaking up the clumps of bacteria and distributing them more evenly throughout the milk, thus effecting a more uniform action, or if the more uniform results were due merely to keeping the organisms in the milk, the following experiment was performed.   A sample of milk was divided into two portions.   One portion served as a control and was left untreated.   The other portion was shaken with sterile glass beads for 15 minutes.   A microscopic examination showed that incipient churning had taken place.   Five tubes of each

portion were submitted to the test in the usual way. Five tubes of each lot were shaken half-hourly while in the water bath. The results are given in table 4. In view of the fact that incipient churning had taken place, it would not be expected that the sweeping action in this portion would be as great as in the normal milk. This experiment shows that the effect of shaking is due in large part to keeping the bacteria in the milk.

In another series of experiments 1 cc. of rennet was added to 200 cc. of the standard methylene blue solution and 1 cc. of this was added as usual to 10 cc. of milk. As controls, tubes were also run with the standard methylene blue solution without the rennet. The rennet milk coagulated within a few minutes. It was impossible to make accurate observations on the long time reducing milks owing to the shrinking of the curd. In the work

TABLE 6
Reduction times when the milk was coagulated by agar

TREATMENT	NUMBER OF TUBES	AVERAGE TIME	VARIATION
Unshaken.............................	18	12:42	3:15
Shaken................................	18	10:40	0:30
Plus agar.............................	9	9:00	0

reported in table 5, 5 tubes were used in each case. The concentrated rennet solution itself reduced methylene blue. Nevertheless it seems unlikely that, in the concentration used in these milks, any appreciable reducing effect would be noticeable. The divergency between the shaken tubes and the rennet tubes was possibly due to the incorporation of oxygen in the former at the moment of reduction.

In the next series of experiments the milk was coagulated by the addition of 1 cc. of sterile 3 per cent agar to each 10 cc. of milk. Results are given in table 6. The tubes tabulated under "Shaken" were incubated undisturbed for the first 9 hours of the test. This may account for the difference between the reduction times of these tubes and of the agar tubes.

To demonstrate that the lessened reduction times were not

due to any reducing action of the agar itself 5 tubes each of 10 cc. portions of the same milk containing varying amounts of the agar were tested with the results as shown in table 7.   In adding agar to the milk one is adding extra oxygen for the same number of bacteria to consume.   A foreign colloid is also being added. In the light of Virtanen's experiments (1924) and of our own, neither of these additions should have any measurable effect upon the reduction time when only 1 cc. of 3 per cent agar is added to 10 cc. of milk.   The following experiment proves that this con-

TABLE 7

The effect on reduction time of adding varying amounts of agar to the milk

AMOUNT OF AGAR SOLUTION	TIME
cc	
1	9:00
2	9:15
3	9:20
5	9:45
10	10:15

TABLE 8

The effect on reduction time of adding agar solution and water to milk

	UNSHAKEN	SHAKEN
	time	time
Whole milk...	0:45	0:45
Agar and milk.	1:10	
Water and milk....................................	1:00	1:00

tention is true.   Milk was divided into 3 portions.   To one part an equal quantity of agar was added.   To another was added an equal quantity of sterile water, while the third was left untreated. The reduction times of a number of duplicate tubes of each of these are given in table 8.

In the next experiment milk was placed in small bore tubes so bent that the central portion of the tubes rested horizontally upon the floor of the bath while at each end a vertical portion extended to the surface of the water.   In the horizontal parts of the tubes the butterfat could rise but a short distance, while in the vertical

parts the usual condition obtained. The milk in all the horizontal portions decolorized in 11 hours and 15 minutes without variation while decolorization in the vertical ends extended over a period from 12 hours and 30 minutes to 16 hours.

An experiment was made in which the butterfat was kept from rising in the milk by homogenization. A fresh milk was run through a clean homogenizer without the application of pressure. The milk was put through again under a pressure of 2500 pounds. It was run through the third time under a pressure of 3500 to 4500 pounds. The usual technique of the reduction test was then used. The results of 15 duplicate tubes in each case are presented in table 9.

TABLE 9

The effect of homogenization on reduction time

	UNSHAKEN		SHAKEN	
	Average time	Variation	Average time	Variation
Unhomogenized......................	10:24	1:00	6:30	0
First homogenization..................	6:45	0	6:49	0:10
Second homogenization................	6:47	0:05	6:47	0:05

It is to be noted that the two means, homogenization and mixing at intervals during the test, of keeping the fat and bacteria from concentrating in the upper layers of the milk have produced the same results.

It has been found that shaking, or any method which keeps the bacteria in the milk, not only frequently shortens the reduction times and wipes out the variations often noticed, especially in good milks, but in all cases causes uniform disappearance of the blue color throughout the whole tube.

These experiments show the sweeping effect of the rising butterfat and provide an explanation for the uneven disappearance of the dye from the individual tubes and for the variations in duplicate tubes. The sweeping effect is less at the bottom of the tube and increases upward. Skar suggests that there may also be a settling of the bacteria and leucocytes to the bottom of the tube.

The effect of the concentration of the bacteria in the upper layers is frequently seen since reduction often occurs here before, or simultaneously with, reduction at the bottom. The cream layer almost invariably decolorizes first. This leaves a portion in the middle of the tube containing, in comparison, few bacteria, thus leaving a blue band in this region. Diffusion of oxygen is necessarily very slow and one portion of the tube has little effect upon distant parts. Since the factors involved are physical and not chemical, duplicate samples will vary in the number of bacteria left in the milk. This accounts for the variations in the reduction times of duplicate tubes and for the white and blue regions and patches so frequently observed. Large variations have been confined almost entirely to good milks of low bacterial content. This is what one would expect since a larger percentage of bacteria is likely to be swept out of such a milk than out of milk of high bacterial content.

Shaking the milk during the period of incubation introduces two sources of error. There is the possibility of the incorporation of oxygen just at the moment of reduction. We found that shaking every 15 minutes or just at the end of the test prolonged the time required for reduction from 15 minutes to 1 hour. In working as much care as possible was taken to avoid this error. In long-time reducing milks this added reduction time is small in comparison to the total reduction period and is much more than counter-balanced by the effect of keeping the bacteria down in the milk. There is also the possibility of the aeration affecting the rate of growth of the bacteria. Little is known regarding this effect. Rogers and Whittier (1927) reported that the level of growth of Str. lactis is higher on aeration. This factor cannot be operative in the cases here reported except, perhaps, during the latter part of the reducing period and would thus be insufficient to exert any appreciable effect. The data here cited are indicative of this.

In order to learn the magnitude of the effect due to creaming on reduction time in milks of varying bacterial content 95 samples of milk were examined. Tubes of each were allowed to remain undisturbed. Duplicate tubes were inverted at 30-minute in-

tervals.   Variations of less than 15 minutes were not considered.
The maximum variation in the case of unshaken tubes was 2:30
on a milk of the 10–12-hour group, in the "shaken" group 15
minutes in one of the 8–9-hour group.   The effect of keeping the
bacteria uniformly distributed in the milk during the test is most
evident from the data of table 10, both as regards constancy of
reduction time and length thereof.   The relation between oppor-
tunity for creaming, due to longer reduction periods, and in-
creased reduction time is evident from the last column of the

TABLE 10

The effect of shaking samples of milk during incubation on length and constancy of reduction time

TIME OF REDUCTION	NUMBER OF SAMPLES	NUMBER OF SAMPLES SHOWING VARIATIONS IN DUPLICATE TRIALS		AVERAGE DECREASE IN REDUCTION TIME DUE TO SHAKING
		Unshaken	Shaken	
hours				*hours*
10–12	5	5	0	−3.00
9–10	5	5	0	−2.5
8–9	8	4	1	−2.0
7–8	10	3	0	−1.1
6–7	8	1	0	−1.5
5–6	9	1	0	−1.5
4–5	10	1	0	−1.4
3–4	11	1	0	−0.7
2–3	7	0	0	−0.25
1–2	11	2	0	−0.20
–1	11	0	0	0.00

table.   Consideration was given to the amount of fat in the milks
tested.   No observations were made which indicate any marked
parallelism between the increased reduction time and high butter-
fat content of the milk.

It does not seem wise to suggest any modification of the test as
described in the last edition of "Standard Methods for Milk
Analyses," 1928.   It is evident that mixing at intervals during
the incubation period will lead to more accurate results with good
milks.   With the poorer milks the effect on accuracy of mixing
would be slight.   It should be remembered that the test is being

used to divide milks of widely varying bacterial content into a few groups, rather than to differentiate between milks of approximately equal bacterial content. The test will be most valuable as a control agent when kept as simple as possible. The technique of mixing should probably be introduced in any attempt to correlate the reduction time of good milks with the results supplied by any other method of determining bacterial content.

THE USE OF A MEASURING DIPPER

"Standard Methods of Milk Analysis" calls for the use of steamed or sterilized 10 cc. pipettes for measuring the samples of milk to be tested. In the routine application of the test this practice has been found to be cumbersome and lessens its simplicity. In some milk distributing plants and cheese factories a 10 cc. dipper has been adopted in the place of the pipettes. At least one manufacturer has such a dipper upon the market.

To determine if such a method is permissible experiments were performed upon a number of samples of milk of all classes. No difference in reduction times could be observed between those samples pipetted and those measured with the dipper. It was found that under careful laboratory conditions such a dipper measured water with less variation than the various 10 cc. pipettes picked up at random in the laboratory. This accuracy, however, is unlikely in the weigh room of a milk plant or cheese factory. Scrutiny of our results on the effect of dye concentration on reduction times (Thornton and Hastings, 1929) will disclose the fact that extreme accuracy in measuring the sample is not necessary. Rather wide variations will give identical results. The reason for this has been explained. Therefore a 10 cc. dipper is sufficiently accurate for use in this test.

STERILITY OF DIPPER AND DYE SOLUTION

If non-sterile apparatus will give the same results as sterile equipment, then the use of the former will add materially to the attractiveness of this test in the mind of the practical man. A study of this question was made and the results of two experi-

ments are given. Two samples of incoming milk were each collected in a quart bottle. A sample was taken out of each for a control. After taking each measureful of these milks the dipper was rinsed twice in a 12-quart pail of cold water, then dipped in a sample of milk which was just sour to the taste and which had a reduction time of less than 5 minutes. The dipper was then rinsed twice in the pail of cold water before being used to measure the next sample. Sixteen samples of each milk were taken which would be the equivalent of collecting 64 samples in the receiving room every other sample being sour. No influence on the reduction time was noted with either milk, all samples of milk 1 reducing in 1:15, of milk 2 in 4:45.

This experiment was repeated using two other milks and 40 samples of each were measured out. The technique of immersing the dipper in a sour milk and rinsing between each sample was identical with the last experiment. This was the equivalent of collecting 160 samples in the weigh-room. No evidence was obtained which indicated that the bacterial content of the samples taken at the end of the sampling was greater than at the beginning.

The conditions under which these tests were made were so much more extreme than one would meet in practice that we are justified in concluding that, in the routine application of the test, it is sufficient to rinse the dipper in the weigh-tank or can of milk about to be sampled if readings are not made after the 5½-hour period. Up to 0.25 gram of milk will cling to a smooth dipper of this size after the ordinary emptying. If this were a milk containing 4 million bacteria per cubic centimeter and were mixed with a can of milk, but 25 bacteria per cubic centimeter would be added to this next sample. This makes the results of our experiments seem reasonable.

A study was made of the effect of sterilizing the methylene blue solution as follows: Sterile dye was added to 30 tubes of milk and incubated. To 30 tubes of the same milk a non-sterile dye was added. The water used for dissolving the dye in this experiment was one not considered fit for drinking purposes. The average reduction time for the sterile dye was 8:58 with a variation between extremes of 45 minutes. The average reduction time for the non-sterile dye was 9:21 with a variation of 45 minutes.

In another experiment 10 tubes of milk contained sterile methylene blue solution and 10 tubes of the same milk contained dye solution which had stood exposed to the laboratory air for one month. The average reduction time for the sterile dye was 5:09 with a variation of 10 minutes. The average for the non-sterile dye was 5:54 with a variation of 20 minutes.

In the routine application of the test the use of any water fit for drinking is warranted as a solvent of the dye if the reduction times are not to be read after the 5½-hour period.

THE PLATE COUNT AND THE REDUCTION TIME

A vast amount of work has been done in attempting to find the correlation between the methylene blue reduction test and the bacterial content of milk as shown by the plate count. Indeed, most of the workers in recent years have done a certain amount of this sort of investigation. The purpose of such investigations has been twofold—to give us a comparative classification of milks and a knowledge of the accuracy of this test.

Those experimenters who regarded the plate method as an approximate one only have been inclined to judge the methylene blue test sufficiently accurate to be of much practical value. Those who have accepted the plate count as something approaching infallibility have doubted the value of the reduction test. The more or less wide spread feeling that these two methods should give almost identical results, failing which one test or the other should be condemned, is unfortunate. The two methods do not measure the same thing. The plate method is the measure of the number of clumps of bacteria in a minute portion of milk which will grow under the conditions imposed in making such an analysis. The methylene blue reduction test is a measure of bacterial activity in milk itself under the conditions imposed in this test. One could not reasonably expect the two methods to agree exactly. Because in many cases they have not agreed, the reduction test has been called a "rough" test. Some seem to fear that an effort is being made to supplant the plate count. Surely these can have small appreciation of the difficulties surrounding milk control. No one method of bacteriological analy-

sis should *supplant* another. One method should *supplement* the other.

At one time or another the reduction test has been expected to be a measure of the numbers, kinds, pathogenicity, and virulence of the bacteria in the milk as well as an indicator for dirt, acidity, and preservatives. It would tax all the methods known to the milk technologist to fulfill such expectations. The confusion in which one finds this subject has not been lessened by the failure of many experimenters to use uniform or standard techniques. Kufferath (1919) used a dye concentration of 1:3000–4000, Fred (1912) 1:10,000, while most of the recent work has been done with a concentration of 1:200,000. The same holds true for plating methods. Fred used Heyden-Nährstoff agar with an incubation period of 12 days. Barthel (1917) used gelatine and incubated the plates at room temperature for 6 days. Many workers neglect to report their techniques.

Jensen, Barthel, Schroeter, and Fred have all proposed classifications based upon the plate count. That accepted in this country is the classification of Barthel and Jensen (1912) and is given in "Standard Methods of Milk Analysis" (1928).

Rahn (1920) thinks this classification is not justified by the results from which it was evolved. In an analysis of Barthel's figures he finds discrepancies in 33 per cent of the cases. Twenty-three per cent of these inaccuracies he attributes to errors in the plate counts and 10 per cent to errors in the methylene blue test. He used the microscopic count as his measure of accuracy. Lind (1920) reported having made comparative tests on 1600 samples with results favorable to the reduction test. Bolling (1924) reported that, of "hundreds" of samples tested, only 1 per cent were placed in different classes by the two methods. The classes he used were those of Barthel just cited. His results seem remarkable. Hastings (1919) concluded

It seems evident from the data submitted that the reduction test determines the number of bacteria as accurately as can be done by any other method. . . . . No one who has had experience with plate culture work would consider it possible to place in the correct order samples of milk, such as are presented, even when working with great care, to say nothing of using the ordinary routine methods.
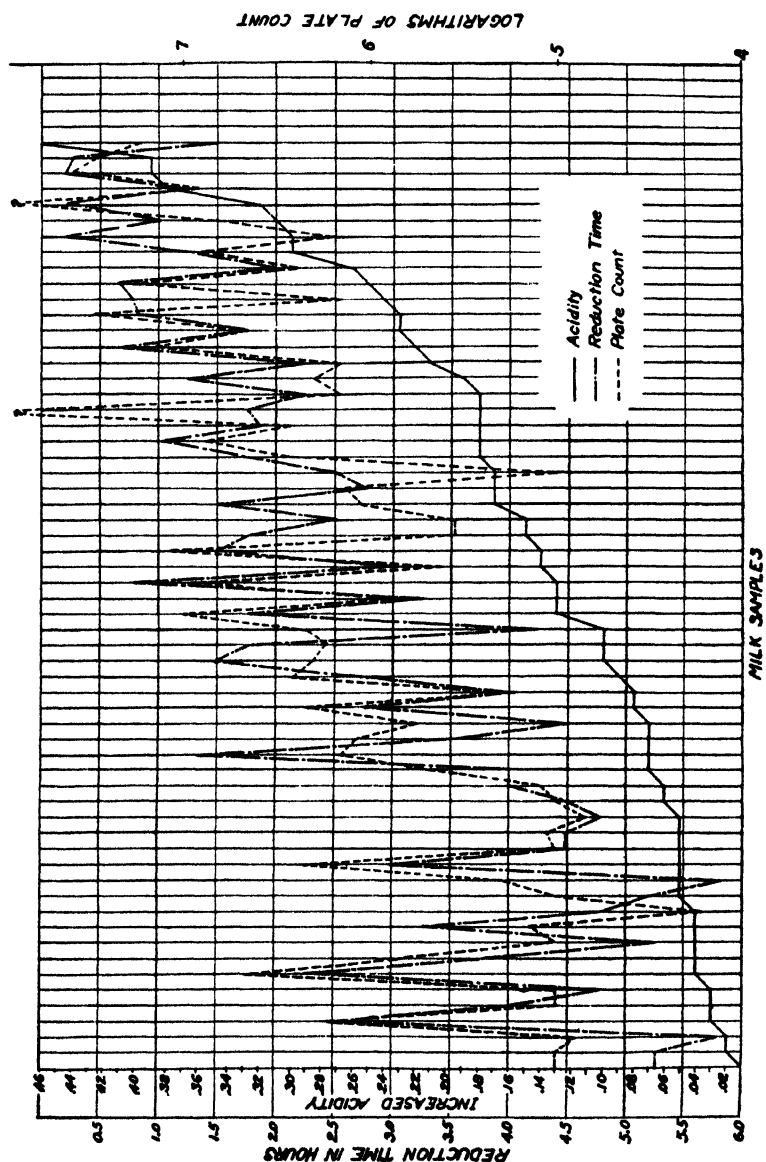
FIG. 1. SHOWING THE RELATIONSHIP OF REDUCTION TIME AND PLATE COUNT TO INCREASED ACIDITY OF 60 SAMPLES OF MARKET MILK

In the development and testing of any method of determining the number of bacteria in milk, attempts have commonly been made to show that the results obtained correlated with those supplied by the plate culture method and with the keeping quality of the milk. The latter is a difficult thing to define or to measure, involving as it does both qualitative and quantitative aspects. The increase in acidity has been the most common measure of keeping quality. This may or may not be a true measure in the case of an individual sample. It is quite certain to be true in general.

The plate count measures a varying fraction of the bacteria present in different samples of milk. The method has certain inherent inaccuracies, the nature and magnitude of which have been the subject of a number of recent papers. A preliminary report on the subject was made by Wright and Thornton (1927). The magnitude of variations noted and a general consideration of the subject of correlation of keeping quality, plate count and reduction time indicated the uselessness of making an extended study of milk to determine the relation of the results obtained by one method to those yielded by another method. The observations which have been made are presented in figure 1, in which the acidity is given in percentage of lactic acid, and the plate counts in the logarithms of the counts. It is to be noted that the trend of the curves is the same in all, with wide variations in reduction time and plate counts from each other and from increase in acidity.

It is probably impossible to divorce the two tests, reduction and plate, in the minds of those engaged in milk control. It will probably be found ultimately desirable to allow each method to stand alone, giving such information as each can to the user. It seems unwise to attempt any closer correlation than is given in the last edition of the "Standard Methods for Milk Analysis" (1928).

SUMMARY

The mechanism of reduction is discussed. The work reported in this paper tends to confirm the theory of Barthel that the disappearance of methylene blue in milk takes place in two stages, viz.:

1. The removal of the dissolved oxygen by bacteria.

2. The reduction of the dye by constituents of the milk.

The time taken for the first stage may be long. The time taken for the second stage is usually short.

The oxygen relationship is of great importance. We were unable to find that a preliminary shaking of the milk materially affects reduction times. Evidence is presented that milks produced normally are in approximate oxygen equilibrium with the atmosphere.

In milks to which this test is applicable, the variations in pH are insufficient to cause a measurable difference in reduction times.

The importance of the rôle played by the leucocytes is not known.

The plate counts of 38 samples of milk taken at the moment of reduction varied from 3.5 million to 45 million with an average of 22 million.

It is probable that the two most important sources of inaccuracy are:-

1. The different rates of oxygen uptake of different species of bacteria.

2. The sweeping of bacteria out of the body of the milk by the rising butterfat during the operation of the test.

It is probable that the inaccuracies in this test follow the growth curve of the bacteria. Therefore, we do not consider the test reasonably accurate after the $5\frac{1}{2}$-hour period as laid down in "Standard Methods of Milk Analysis."

Since the methylene blue is, indirectly, an indicator for oxygen, different concentrations of the dye, within limits defined in a previous paper, give identical reduction times. A 10-cc. dipper is, therefore, sufficiently accurate for use in measuring samples for this test.

It is sufficient that this dipper be thoroughly rinsed in the incoming milk or in water between each sample, if times are not to be read after the $5\frac{1}{2}$-hour period. The use of any water fit for drinking purposes is warranted as the solvent for the dye.

The methylene blue reduction test is as accurate a measure of

the keeping quality of milk as any method yet available. It will divide milks into three or four classes with reasonable accuracy, as will any of a number of tests for milk quality. It is inexpensive and as nearly fool-proof as any method for this purpose available to the dairy bacteriologist. Methylene blue reduction times should not be reported in terms of the plate count.

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STUDIES ON THE NUTRITIVE VALUE OF MILK

IV. THE SUPPLEMENTARY VALUE OF YEAST IN NUTRITIONAL ANEMIA OF ALBINO RATS*

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In attempting to study the nutritive value of milk obtained from various sources by the exclusive milk method of feeding, nutritional disaster, including anemia, was encountered in rats at such an early age that no reliable data as to growth could be obtained. A systematic search for possible clues was undertaken, the results of which were previously reported (1). Various substances known to carry the essentials of a complete diet were added singly to the milk diet. As a source of the vitamin B-complex, yeast from our stock supply was used. The rats receiving this thrived; growth was excellent and hemoglobin remained at a high level. This could not be attributed to vitamin B (complex) as it had been previously shown that 16 cc. of milk was adequate when fed in addition to a basal ration free from this factor and the rats here were' fed milk ad libitum. Furthermore, rice polishings, also supplying the B-complex, were not beneficial. Moreover, the ash of the same yeast was equally effective, suggesting that the factors involved were inorganic in nature.

Since iron had always been associated with hemoglobin, an analysis of the yeast for iron was made. The publication of the work of Hart, Steenbock, Waddell and Elvehjem (2) at this time suggested copper as a factor in hemoglobin formation. Analysis of the yeast for this element was also made. The sample was found to contain 0.0923 per cent iron and 0.0032 per cent copper.

This meant that the daily intake from 0.4 gram of yeast was 0.3692 mgm. of iron and 0.0128 mgm. of copper.

Figure 1 shows the effect of the addition of 0.4 gram of stock dried yeast to an exclusive milk diet, both as a prophylactic and a
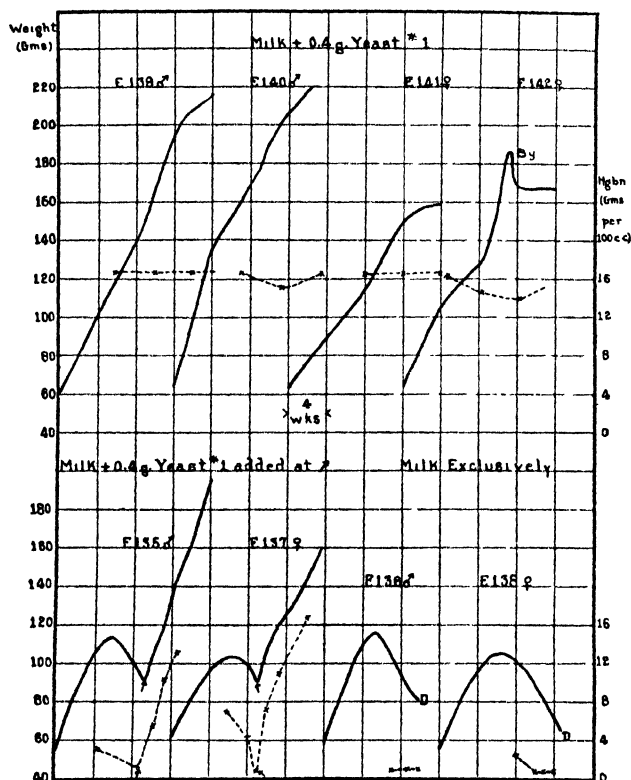
Fig. 1. The remarkable prophylactic and curative properties of yeast 1 are shown in this chart. This sample was rich in iron (0.0923 per cent) and contained a fair amount of copper.

In this and succeeding charts the solid lines signify weight and the broken lines hemoglobin.

curative substance. The curves of two representative rats not receiving the supplement are given for comparison.

The stock supply of yeast having become exhausted, a new supply was obtained from the same source. When 0.4 gram of

this supply was used to supplement milk the results were quite
different from those obtained with the first supply. Growth was
excellent, but the hemoglobin level became lower and lower until
the rats were anemic. Analyses showed 0.0178 per cent iron and
0.0035 per cent copper, considerably less iron than in the first
sample, and about the same amount of copper. Figure 2 shows
the difference in the effect of these two samples on the hemoglo-
bin of representative rats, both sets of curves being compared
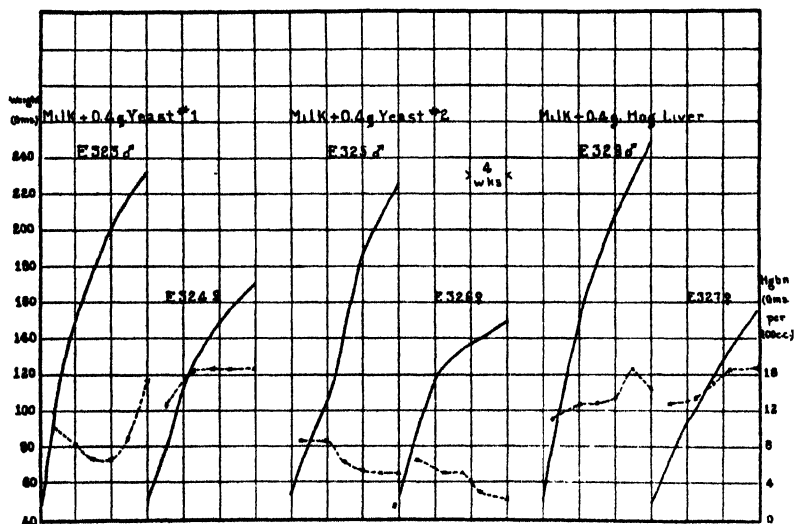
Fig. 2. These curves are representative of a number showing the difference in
antianemic potency of two samples of yeast from the same source. The chief
difference in chemical composition existed in the iron content.

with the curves for rats receiving an equal weight of dried hog
liver.

A third sample of yeast from the same source having given still
different results lying between those of the first two samples, it
was decided that before any conclusion as to the value of yeast in
general as an antianemic substance could be made samples from
various sources must be assayed. Consequently a number of
samples were obtained from the leading manufacturers of yeast
and their antianemic potency determined.

The technique used in making the hemoglobin determinations, and the routine procedure of feeding, weighing, and caring for the rats have already been described (3).   Ten samples of yeast were obtained from 6 manufacturers.   Most of the samples were received in the dry form.   One sample was delivered to the laboratory in fresh cakes, and another arrived in the form of a
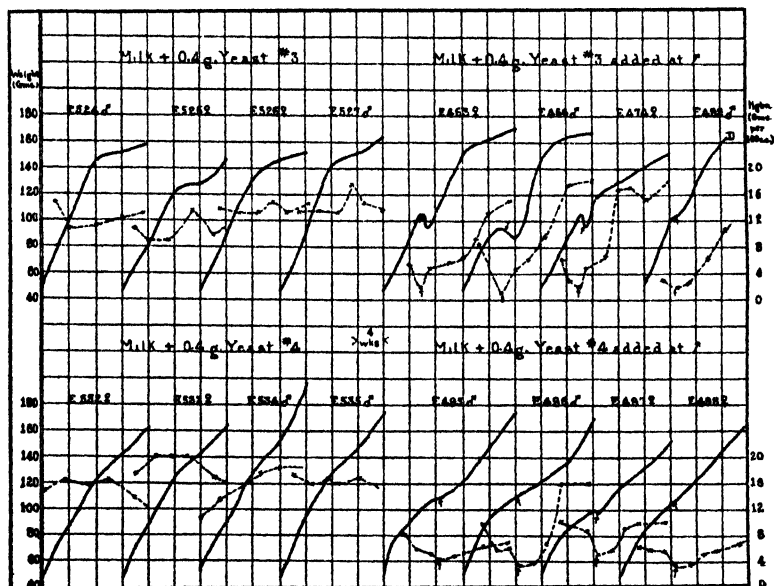
Fɪɢ. 3. Yeast 3 came from the same source as yeasts 1 and 2.   It contained more iron than yeast 2 and more copper than either 1 or 2.

paste.   These were dried and pulverized.   Each sample was analyzed for dry matter, total nitrogen, ash, copper, and iron. The Official Methods were used for dry matter, total nitrogen, and ash.   The Biazzo method (4) was used for copper, and the method of Fowweather (5) for iron.

Each sample was tested as a prophylactic and as a curative. In the prophylactic assay, 0.4 gram of dried sample was added daily to the milk from the beginning of experimental feeding.

The experiment continued for 16 weeks. In the curative assay the hemoglobin of the rats was allowed to drop to from 2 to 4 grams per 100 cc. of blood, at which time 0.4 gram of yeast was added daily. The fortified ration was continued until 16 weeks had elapsed from the beginning of the experiment. Hemoglobin determinations were made as a rule every 2 weeks. In some cases where a critical condition seemed imminent hemoglobin determinations were made oftener.
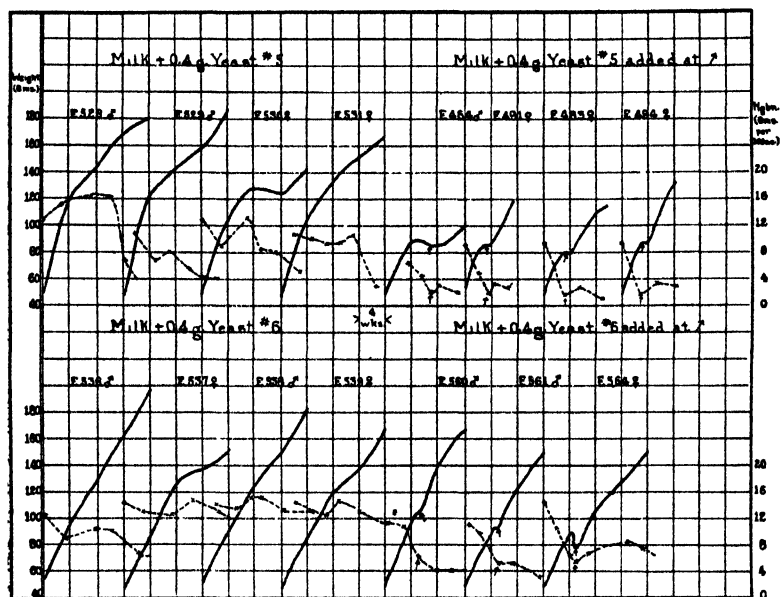
FIG. 4. Yeasts 5 and 6 came from the same company but were prepared in different ways. No. 5 contained less copper than any other sample and gave the poorest results. It was grown in a pure malt wort. No. 6 was grown in molasses wort, with ammonium salts.

RESULTS

The antianemic potency of the various samples of yeast is shown graphically in figures 3 to 6. With 2 exceptions the yeasts showed some potency, the degree of effectiveness varying considerably. In general, they were more effective as prophylactics than as curatives. This was taken into consideration in giving
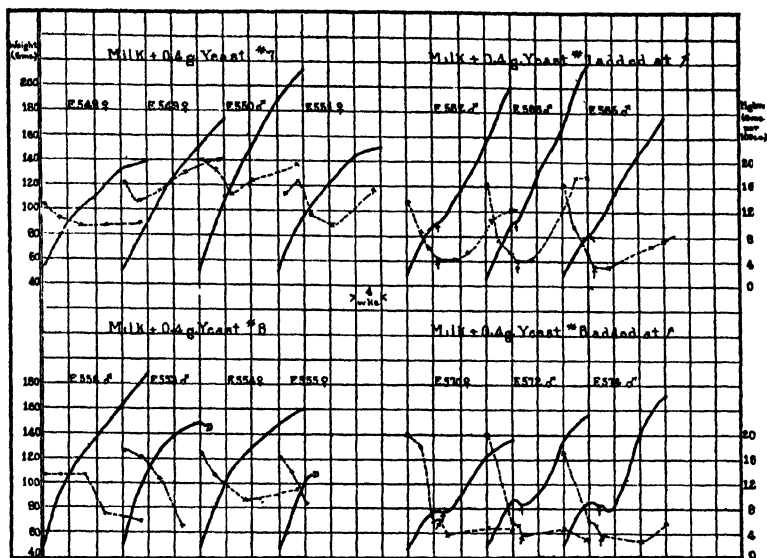
Fig. 5. Yeasts 7 and 8 came from the same company, but were prepared in different ways. No. 7 was grown on grain extract, while No. 8 was grown on molasses. No. 7 contained more copper than any other sample.
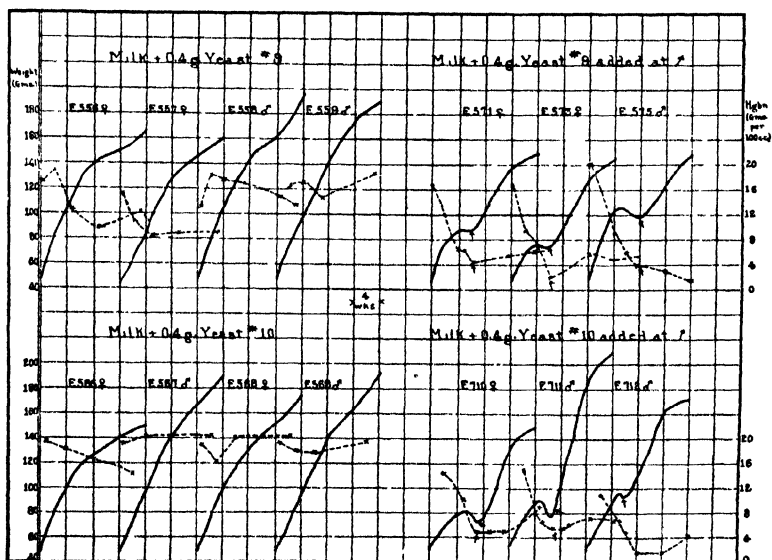
Fig. 6. Yeast 9 was low in both iron and copper. Yeast 10 was low in iron These samples were much more potent as prophylactics than as curatives.

TABLE 1

Analysis of yeasts (per cent)

NUMBER	DRY MATTER	TOTAL NITROGEN	ASH	COPPER	IRON
1	97.04	7.525	12.550	0.0032	0.0923
2	95 78	7.410	9.975	0.0035	0.0178
3	95.92	8.335	11.880	0.0041	0.0281
4	92.81	8.730	6.980	0.0045	0.0153
5	90.09	8.200	11.790	0.0022	0.0193
6	95.78	8.085	6.540	0.0038	0.0186
7	90.42	4.215	6.130	0.0056	0.0154
8	88.53	6.870	8.805	0.0034	0.0194
9	92.26	6.870	7.360	0.0026	0.0086
10	92.43	9.310	6.160	0.0046	0.0142

TABLE 2

Relation between copper content and antianemic potency

NUMBER	PER CENT COPPER	ANTIANEMIC POTENCY
7	0.0056	Very good
10	0.0046	Good
4	0 0045	Good
3	0.0041	Very good
6	0.0038	Fair
2	0.0035	Poor
8	0.0034	Very poor
1	0.0032	Excellent
9	0.0026	Fair
5	0.0022	Very poor

TABLE 3

Relation between iron content and antianemic potency

NUMBER	PER CENT IRON	ANTIANEMIC POTENCY
1	0.0923	Excellent
3	0.0281	Very good
8	0.0194	Very poor
5	0.0193	Very poor
6	0.0186	Fair
2	0.0178	Poor
7	0.0154	Very good
4	0.0153	Good
10	0.0142	Good
9	0.0086	Fair

the final rating to each sample. Yeasts 3 and 7 were particularly effective. Yeast 5 was practically without effect, while yeast 8 exhibited very weak hemoglobin regenerating power. Table 1 shows that yeasts 3 and 7 were rich in copper, that yeast 5 contained the least copper of any sample, while yeast 8 contained fair amounts of both copper and iron.

In order to see if any relationship could be shown to exist between the copper and iron content of the yeasts and their antianemic potency, the various samples are listed in table 2 in descending order according to their percentage copper content and classified according to their effectiveness as shown by the charts. In table 3 they are treated similarly according to their percentage iron content. With one notable exception the samples at the top of the list in table 2 are those that showed marked effectiveness. In table 3 no relationship between iron content and antianemic potency is apparent.

DISCUSSION

The results obtained in this study show that yeasts vary considerably as to chemical composition. This may be due to the nature of the media, the vessels in which the yeasts are grown and the materials with which they come in contact during subsequent treatment.

It is likewise shown that yeasts from different sources vary considerably as to antianemic potency. In trying to link this up with their variation in chemical composition it becomes strongly indicative that it is rather closely related to their copper content. The superior value of yeasts 1 and 3 might be due to the high level of iron intake resulting from their administration. If the theory of the catalytic action of copper on iron is upheld this would seem to be a satisfactory explanation as it could be argued that the lower levels of iron intake had not reached the saturation point. On the other hand, yeast 7 furnished a relatively small amount of iron, but was richest of all the samples in copper, and showed strong hemoglobin regenerating power. This would indicate a direct utilization of the copper. However, in a complex substance such as yeast there are other inorganic substances

which were not determined and which might have exerted an influence.

Titus, Cave and Hughes (6) have obtained excellent results in hemoglobin regeneration through the use of manganese, while Myers and Beard (7) have obtained responses through the use of rarer elements, such as germanium, nickel, and cobalt. In view of the work of Waddell, Steenbock, Elvehjem and Hart (8), McHargue, Healy and Hill (9) and Krauss (10) the part played by copper, as shown in table 2, is outstanding.

Since the completion of the experimental work presented here, Sure and Kik (11) reported beneficial effects in hematopoietic function through the use of yeast. They found a greater hemoglobin concentration in nursing young when the mothers received a diet containing 10 per cent of dried yeast than when they received the stock diet. Sure, Kik, and Walker (12), referring to some of their own unpublished data, state that during gestation they find no anemia on synthetic diets containing an abundance of wheat embryo or yeast as sources of the vitamin B complex. In a later publication, Sure, Kik, and Walker (13) state that "the character of our results certainly does not show any relation between a deficiency of the vitamin B complex or uncomplicated vitamin B deficiency and pernicious anemia, or, as a matter of fact, does not establish the association with any definite form of anemia." It would seem, then, that the hemoglobin-regenerating power shown by certain samples of yeast is in no way related to their richness in the factors of the vitamin-B complex.

That the addition of yeast to an exclusive milk diet may exert some benefit other than in hemoglobin regeneration is indicated in the charts where it is shown that growth continues even after the hemoglobin has reached a low level. This may be due to greater food consumption and to an adequate intake of vitamin B. On an exclusive milk diet the growth curve follows the hemoglobin curve. Milk consumption decreases and, since milk has been shown to be a poor source of vitamin B (14), this may limit growth.

The iron requirements of infants on an exclusive milk diet are well known. If in the human, as in the rat, copper is a stimulus

for hemoglobin formation, some suitable substance furnishing both iron and copper should be administered. Hoobler (15) has fed yeast to infants with good results. This may have been beneficial not only because of the additional vitamin B (complex) furnished, but because of the increased intake of copper and iron.

SUMMARY

Ten samples of yeast from various sources were assayed for antianemic potency. Seven samples showed definite hemoglobin regenerating power of varying degrees. Three samples showed very little or none.

Some correlation appeared to exist between the copper content of the yeasts and their antianemic potency. No such correlation was evident with respect to iron. Other substances, recently claimed to be concerned with hemoglobin regeneration, were not considered.

Some supplementary effect on growth was shown by the addition of yeast to milk. This was attributed to vitamin B.

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LENGTH OF GESTATIONS IN JERSEY COWS*

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Duration of pregnancy is a problem ever confronting breeders of dairy cattle, for it is absolutely essential in the management of livestock for the owner or herdsman to have a definite knowledge as to when his pregnant cows are due to calve. The length of the gestation period is therefore of interest not only to the student of embryology but to every farmer who owns or cares for livestock. It is regrettable in view of the importance of the subject that the literature pertaining to the length of gestations is very meagre.

In recent years very few results of investigational work dealing with gestations in dairy cattle have appeared in print. Numerous gestation charts or tables have been published showing easy methods of figuring the "approximate" calving date. These tables do not all agree, some being based on gestations of 40 weeks, some on the basis of 282 days and others for shorter periods of time.

In 1817, M. Tessier (1) studied 575 cases and reported a variation from 241 to 321 days. The average was 282.2 days and 95 per cent of the calves were carried from 270 to 299 days. In a more extensive tabulation made by the Earl of Spencer (2) in 1840, the period varied between 220 and 313 days for calves born alive. The average was 283.3 days for 764 cases, including 23 twin births which averaged 277.2 days. About two-thirds (67 per cent) were from 280 to 289 days, 90 per cent were from 275 to 294 days and 95 per cent from 270 to 299 days. None born under 242 days were raised.

C. B. Bement (3), in 1845 found the variation to range between 276 and 299 days except for one short period of 213 days and a long one for 336 days. The average was 286 days (288 for 36 bull calves, and 283 for 26 heifers). Various breeds, Shorthorns,

Devons, Herefords, Ayrshires, and grades were included. L. F. Allen (4) in 1868, reported a study of 50 cows of different breeds showing an average gestation of 284 days, with a range of 268 to 291 days.

H. H. Wing (5) tabulated 182, cases, mostly Holsteins and Jerseys. The range in the length of the gestation periods was from 264 to 296 days. The average found was lower than reported by the Earl of Spencer or Tessier being 280 days for 97 Holsteins and 279 days for 56 Jerseys. Five twin births averaged 275 days. Fitch, McGilliard and Drumm (6) in a study including four dairy breeds reported that the breeds varied in length of gestation as follows:

		days
Jersey	—100 gestations	284.3
Guernsey	—103 gestations	283.0
Ayrshire	—113 gestations	284.6
Holstein	—220 gestations	281.0

The average length of all the gestations studied was 282.4 days. The bull calves were carried 283.2 days and the heifers 281.9 days.

INVESTIGATIONAL

When a Register of Merit record is completed a calving affidavit is sent to the owner. It is·retained by him and if the cow drops a living calf after finishing her test period, he fills in both the service and calving dates. The accuracy of these dates and the fact that the calf was alive at birth must be attested before a notary and then the affidavit is returned to the Breed Association office. If the cow does not calve within a certain length of time or if the calf is not alive at birth no affidavit is necessary.

This study concerns the records accepted by the American Jersey Cattle Club during 1927 for which calving affidavits were returned swearing that living calves were dropped. It included 1075 gestations, all of which were apparently normal, if a living calf can be assumed as evidence of normality. The length of each of these gestation periods was determined and also the sex of the calf in all cases where the progeny had been registered. In

computing the length of the gestation period the day on which the calf was born was included in the total number of days, but not the day of service. For example, if a cow was served on January 12 and calved on October 18 the length of the period of pregnancy would be considered as 279 days. These gestations were then grouped according to the age of the dam at the time of calving. In table 1, are presented the data covering this phase of the investigation.

The shortest gestation reported was for 228 days and the longest for 312 days. Approximately 62 per cent of all the periods

TABLE 1

Effect of age in length of gestation periods

AGE	NUMBER OF CASES	AVERAGE LENGTH OF GESTATIONS
Yearlings and Jr. 2.........	141	278.51
Sr. 2	46	277.96
Jr. 3	195	278 34
Sr. 3...	124	278.63
Jr. 4.........	111	278.38
Sr. 4	100	278.80
5	142	278.73
6.....	89	277.45
7...	58	279.26
8..	27	277.56
9	17	280 35
10	12	280.58
11	6	277 67
12 years and over.........	7	281.43
Total	1.075	278.51

fall between limits of 275 and 283 days and 81 per cent come between 271 and 285 days. The average length of all the gestation periods was 278.51 days. However, only 68 of the 1075 calves were born on the 278th day of pregnancy, and the data indicate that there is almost an equal chance of the calf being born on any day after 271 days have elapsed and until the 285th day is reached. Table 2 illustrates how these gestations group themselves when arranged according to their length.

At the time this study was made, only 683 of the progeny had been registered in the Herd Books of the American Jersey Cattle Club. Of this number 297 were bull calves, 382 heifers and 4 sets of twins. On the basis of sex, the gestation periods of the 297 bull calves averaged 279.48 days and the 382 heifers averaged 278.64 days. The 4 sets of twins showed an average gestation of 275.50 days.

It was also of interest to find that while 63 per cent of the 1075 calves were registered, that of the 26 carried for 265 days or less only 11 or 42 per cent were registered while the 44 calves carried

TABLE 2

Frequency table grouping gestations according to length

CLASS	FREQUENCY
250 days and below	5
251 to 255	4
256 to 260	10
261 to 265	7
266 to 270	46
271 to 275	206
276 to 280	384
281 to 285	325
286 to 290	61
291 to 295	19
296 to 300	6
Over 300 days	2

288 days or longer, 30 or 68 per cent were registered. Of the 5 calves carried for less than 250 days only one was raised, and of the 8 carried for 296 days or more, 5 were raised. This would indicate that those calves resulting from abnormally long gestations have a better chance of living than do those born prematurely.

Another question is whether certain cows do not possibly have a habit of carrying their calves either for shorter or longer period of time than the average for the breed. That is, if a heifer drops her first calf at 270 days, should the owner expect her following gestations to be shorter than usual. Twelve of those cows showing gestation periods longer than 290 days had at least 2 additional

recorded progeny and the same was true of 13 of the cows with gestations of 265 days or less. The life time breeding records of

TABLE 3

NAME OF COW	LENGTH OF GESTATIONS	SEX OF CALF	NAME OF COW	LENGTH OF GESTATIONS	SEX OF CALF
Progeny records of twelve cows carrying at least one calf for an abnormally long gestation period

NAME OF COW	LENGTH OF GESTATIONS (days)	SEX OF CALF	NAME OF COW	LENGTH OF GESTATIONS (days)	SEX OF CALF
Flying Chief's Golden Beauty 480952	275	F.	Queen Ladette of Bryan 628569	281	F.
	281	M.		284	M.
	276	M.		295	M.
	280	F.			
	282	M.	Oxford's Royal Nora 595773	279	M.
	291	M.		287	M.
Tom's Lady Capitan 345664	280	M.		286	M.
	280	F.		290	F.
	279	M.	Design's Dairylike Ildg 663202	273	M.
	280	F.		276	F.
	280	M.		299	F.
	280	F.			
	299	*	Campanile's Pansy 585974	280	F.
	281	M.		281	F.
	278	M.		285	F.
Able Fox's Rainbow 566179	273	F.		291	F.
	275	F.			
	280	M.	Belle's Huckleberry 521808	279	M.
	293	M.		274	F.
				296	*
Miss Dumont's May 566269	280	F.	Knight's Dolly Dimple 510442	289	F.
	279	F.		282	F.
	280	F.		282	M.
	289	M.		285	M.
	310	M.		297	F.
Fomasa's Irene 550669	282	F.	Sir Owl's Jewel's Julia 640298	272	F.
	282	F.		292	F.
	290	*		279	M.

' Not registered.

TABLE 3—*Continued*

NAME OF COW	LENGTH OF GESTATIONS	SEX OF CALF	NAME OF COW	LENGTH OF GESTATIONS	SEX OF CALF
Progeny records of thirteen cows carrying at least one calf for an abnormally short gestation period					
	days			days	
Passport's Jap's Princess 616755	281	F.	Jap's Poetess 460418	283	F.
	266	M.		281	F.
	261	F.		290	F.
				237	*
The Kid of Ohio's Gusta 654807	279	F.	Prince's Louise M 494446	279	M.
	259	*		290	M.
	278	M.		231	*
Majesty's Hildegarde 558997	279	F.	Golden Fontaine You'll Do 664077	280	F.
	278	F.		280	M.
	228	*		265	*
Sovictor Lenore 543955	279	M.	Imp. Beaulieu Comtesse 692510	283	M.
	285	M.		275	M.
	264	F.		251	M.
Dolly's Milkmaid 575423	279	M.	Tiddledywink's Princess Eva 645558	282	F.
	284	F.		256	*
	265	M.		282	M.
Star's Jolly T. A. 598467	282	M.	Owl Knight's Lady 526650	280	F.
	278	F.		280	F.
	282	F.		281	F.
	281	F.		281	M.
	256	F.		239	*
Jewel's Majestic Betsy 588297	273	F.		279	F.
	285	F.			
	257	F.			
Jersey one-thousand-pound fat producers with four or more registered progeny					
Abigail of Hillside 457241	277	F.	California's Rinda's Insie 565559	280	F.
	284	M.		271	F.
	282	M.		278	F.
	277	M.		276	M.

* Not registered.

TABLE 3—*Continued*

NAME OF COW	LENGTH OF GESTATIONS	SEX OF CALF	NAME OF COW	LENGTH OF GESTATIONS	SEX OF CALF

Jersey one-thousand-pound fat producers with four or more registered progeny—*Continued*

NAME OF COW	days	SEX OF CALF	NAME OF COW	days	SEX OF CALF
Darling's Jolly Lassie 435948	285	M.	Lad's Iota 350672	292	M.
	277	M.		266	M.
	277	F.		275	F.
	281	F.		277	F.
	286	M.		272	F.
	280	F.		254	F.
	278	F.		274	M.
	281	M.			
Prince's Emma of H. S. F. 359390	276	M.	Madeline of Hillside 389336	278	F.
	281	F.		280	M.
	284	F.		278	M.
	277	F.		284	M.
	274	F.		285	M.
Lady's Silken Glow 313311	275	F.	Plain Mary 268206	282	F.
	286	M.		287	F.
	278	F.		284	F.
	281	F.		288	M.
	284	M.		268	M.
				288	M.
				274	F.
St. Mawes Lad's Lady 451568	252	F.	Vive La France 319616	275	M.
	276	M.		265	F.
	278	M.		277	M.
	283	M.		276	M.
	272	F.		271	F.
	278	F.		286	M.
	279	M.		284	M.
Red Lady 396118	281	M.	Fauvic's Star 313018	273	F.
	276	F.		285	M.
	279	M.		280	F.
	282	F.		273	F.
	275	M.		259	M.
	282	F.			
	282	F.			
	284	M.			

TABLE 3—*Concluded*

NAME OF COW	LENGTH OF GESTATIONS	SEX OF CALF	NAME OF COW	LENGTH OF GESTATIONS	SEX OF CALF
Jersey one-thousand-pound fat producers with four or more registered progeny—*Concluded*					
	days			*days*	
Princess Elise 418113	271	F.	Princess Xenia 356699	282	F.
	278	M.		280	F.
	276	F.		278	F.
	285	F.		281	M.
	285	F.			
St. Mawes Lad's Pride 515044	273	F.	Tiddledywink's Quality Girl 560784	279	F.
	267	F.		279	F.
	272	M.		278	M.
	295	M.		277	M.

these 25 cows were studied. Also, investigation showed that 17 of the 24 Jersey cows with butterfat records over one thousand pounds of fat were the dams of 4 or more registered calves. It was believed that the breeding records of such cows would be the most reliable obtainable and the gestation periods of their progeny are therefore given in table 3.

Examination of the breeding records of the cows in these groups does not reveal any correlation between the length of pregnancies for the same individual dam. In fact a great deal of variation is noted in most cases. In the first group the 12 extremely long gestations averaged 295.25 days in length and the other 40 gestations of these same cows averaged 280.15 days. In the second group the 13 abnormally short gestations averaged only 251.46 days in length while 32 other gestations by these 13 dams averaged 280.47 days. From these records it does not seem possible to predict or determine the probable length of a future gestation period even though the length of several previous periods are known. Included in the 3 preceding groups are 186 gestations. The average length of all of these gestations is 278.19 days. However, since the accuracy of the service dates of these preg-

nancies have not been attested to they were not included in the 1075 gestations on which the main portion of this study was based.

SUMMARY

1. The average gestation period for the 1075 cases studied was 278.51 days but there appears to be an equal chance of a cow calving anytime between the 271st and the 285th day.

2. The age of the dam apparently has no effect on the length of gestation.

3. Bull calves were carried an average of one day longer than heifer calves, 297 bull calves being carried for an average of 279.48 days and the gestations of 282 heifer calves averaging 278.64 days.

4. The data are too scant to draw conclusions relative to twin births but the results compare with those obtained by other investigators in that the gestations of twins are usually several days shorter than the average.

5. Calves resulting from abnormally long gestations seem to have a better chance of living than do those born prematurely.

6. No correlation could be detected between the length of different gestations in the same individual dam. Cows showing one or more short gestations also exhibited other gestations longer than the average and vice versa.

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QUANTITATIVE CHANGES IN THE MICROFLORA OF BUTTER DURING STORAGE*

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In connection with studies concerning factors which influence the market quality of butter, data have been accumulated on the mold, yeast and bacterial counts of butter kept in storage for various periods of time and at different temperatures. These data are presented in the belief that they may be useful in the interpretation of changes occurring in the microflora of butter.

Several lots of butter were available for study. Lot 1 represented 63 samples of salted butter manufactured in 8 different creameries in Minnesota during August, 1927. This butter was in 1 pound prints wrapped and stored at the creamery for 1 to 6 days at 30° to 45°F. and in transit at 42° to 67°F. for 7 to 8 days to the eastern markets through regular channels. Lot 2 consisted of 55 samples of salted butter manufactured at the Minnesota State Experimental Creamery during the period of one year (1926–1927), packed in 3 to 5 pound stone jars and kept for 1 month at 32° to 35°F. Lot 3 was made up of 68 additional samples of salted butter from the same creamery, packed in jars and stored at 32° to 35°F. for 9 months. Lot 4 consisted of 297 samples of salted butter, mostly in 20 pound tubs, manufactured in various creameries throughout the country and entered in the 1927 storage contest of the National Creamery Buttermakers' Association. The butter was stored at −5 to −10°F. for a period of 5 months. Lot 5 represented 55 samples of unsalted butter from the same churnings as lot 2, stored in the same type of package, and for the same period. Lot 6 was made up of 68 samples of unsalted butter from the same churnings as lot 3 and kept under the same conditions. Thus, 483 samples of salted and 123 samples of unsalted butter were available for study.

Mold, yeast and bacterial counts were made for all samples before and after storage.   In the case of lots 1, 2, 3, 5, and 6 counts were made immediately after the butter was made while in the case of lot 4, the analyses were made at the time the butter went into storage, which meant that the butter was from a few days to a few weeks old before samples were available.   All counts of storage butter were made when the butter was taken from storage or when it reached the market as in the case of lot 1. Samples were taken and platings made in the usual manner. Whey agar served as the medium, and in the case of mold and yeast counts, the agar was acidified with a tartaric acid solution previous to plating.   Mold and yeast counts were made after incubation at 20° to 25°F. for 5 days unless plates were crowded in which case, counts were made at the end of 2 or 3 days.   Bacterial counts were made after the plates had been incubated at 20° to 25°C. for 5 days and an additional period of 2 days at 37°C. All counts are expressed in terms of the number of colonies per cubic centimeter of sample.

When the data were assembled it was found that the results obtained with lots 1, 2, 3 and 4 were so generally consistent that it was not considered necessary to include, in this report, the results from each individual lot of butter but to present the combined results representing all samples of salted butter.   The same situation was true of the results from the two lots of unsalted butter, consequently the figures given represent all unsalted samples.   The differences in the results obtained under different temperatures and periods of storage were not pronounced and did not influence the general trend.

The data presented in table 1 show the range in mold, yeast and bacterial counts for all samples, before and after storage.   It will be noted that the majority of mold counts of fresh butter, salted or unsalted, are below 10 per cubic centimeter.   After storage a greater proportion of the salted butter had such a low count

while the proportion of low counts in the unsalted butter had diminished, so that the major portion of counts were in the hun-

TABLE 1

Distribution of counts in fresh and stored, salted and unsalted butter

	DISTRIBUTION OF COUNTS			
COUNTS	Before storage		After storage	
	Salted	Unsalted	Salted	Unsalted
Molds per cubic centimeter	*per cent*	*per cent*	*per cent*	*per cent*
0–10	73.5	61.8	88.0	22.8
11–50	18.6	24.4	7.3	10.6
51–100	2.1	5.7	0.8	2.4
101–500	2.9	3.3	1.6	14.6
501–2,500	1.2	3.3	2.1	10.6
2,510–10,000	1.5	1.5	0.0	15.4
10,100–50,000	0.2	0.0	0.0	11.4
51,000–250,000	0.0	0.0	0.2	4.9
251,000–1,000,000	0.0	0.0	0.0	6.5
1,100,000–5,000,000	0.0	0.0	0.0	0.8
Yeasts per cubic centimeter				
0–10	36.4	53.8	47.4	25.2
11–50	25.7	23.5	21.3	11.8
51–100	7.9	2.5	6.6	14.3
101–500	14.7	12.6	10.1	16.8
501–2,500	10.6	5.9	10.1	21.0
2,510–10,000	2.9	1.7	3.5	6.7
10,100–50,000	1.4	0.0	0.4	3.4
51,000–250,000	0.4	0.0	0.3	0.8
251,000–1,000,000	0.0	0.0	0.3	0.0
Bacteria per cubic centimeter				
0–1,000	9.7	17.2	23.4	1.5
1,010–10,000	27.1	27.0	32.3	5.7
10,100–50,000	25.3	13.1	25.7	10.7
51,000–250,000	21.1	9.8	9.9	19.6
251,000–1,000,000	9.9	13.1	6.0	26.2
1,100,000–10,000,000	6.9	15.6	2.7	27.9
11,000,000–66,000,000	0.0	4.2	0.0	8.4

dreds or thousands. This clearly shows the influence of salt upon the molds.

In considering the yeast count, one finds that the majority of

counts of the salted butter are below 50 per cubic centimeter.   It is quite to be expected that the general level of yeast counts will be higher than the mold counts as previous data have consistently indicated.   It is evident that the differences between the counts of fresh and stored butters are not as marked as they were in the case of the mold counts.   The effect of salt is manifest but not as decided as it is for molds   It has been generally found that yeasts are not as sensitive to higher salt concentrations.

The bacterial counts of the fresh butter are largely below 50,000 although a considerable proportion of the fresh unsalted butter had bacterial counts in the millions per cubic centimeter.   The

TABLE 2

Change in counts during storage of butter

	KIND OF BUTTER	TOTAL SAMPLES	DISTRIBUTION OF SAMPLES SHOWING		
			Increase	No change	Decrease
			per cent	*per cent*	*per cent*
Molds.........................	Salted	483	22.6	12.6	64.8
	Unsalted	123	85.4	4.0	10.6
Yeasts........................	Salted	483	32.3	5.4	62.3
	Unsalted	119	71.4	3.4	25.2
Bacteria......................	Salted	483	25.3	1.0	73.7
	Unsalted	122	72.1	0.0	27.9

effect of salt is very marked in the case of the bacteria according to the data presented.   A markedly increased proportion of the bacterial counts of salted butter are below 50,000 per cubic centimeter at the end of the storage period while the counts of the unsalted samples after storage are largely in the realm of millions. The influence of salt is much more marked than it was for yeasts or molds.

To approach the situation from another angle, consider the data presented in table 2 which show the relative increases or decreases in count during storage.   The outstanding feature of these data is the marked differences between the changes taking

place in the salted and unsalted butter. The fact that 70 to 85 per cent of the unsalted samples increase in count during storage, while 60 to 75 per cent of the salted samples decrease is altogether in accordance with expectation and is in line with data previously available in the literature.

TABLE 3

Ratio of increase or decrease in count during storage of butter

(Ratio of decrease—Original count : final count :: x : 1)

(Ratio of increase—Original count : final count :: 1 : y)

RATIO	DISTRIBUTION OF SAMPLES ACCORDING TO RATIO OF CHANGE IN					
	Mold counts		Yeast counts		Bacterial counts	
	Salted butter	Unsalted butter	Salted butter	Unsalted butter	Salted butter	Unsalted butter
Ratio of decrease (x:1) *(x = original count)*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
100,001–250,000:1	0.0	0.0	0.0	0.0	0.2	0.0
25,001–100,000:1	0.0	0.0	0.0	0.0	0.4	0.0
5,001–25,000:1	0.0	0.0	0.0	0.0	0.4	0.0
1,001–5,000:1	0.2	0.0	0.4	1.6	0.2	0.7
251–1,000:1	0.9	0.0	0.6	0.0	1.7	1.6
51–250:1	1.1	0.9	3.5	2.5	7.7	4.1
11–50:1	9.9	2.4	10.8	8.4	20.0	7.4
1–10:1	52.8	7.3	47.0	12.6	43.0	13.9
0	12.8	4.1	5.4	3.4	1.0	0.0
Ratio of increase (1:y) *(y = final count)*						
1:1–10	19.9	22.0	25.1	24.4	22.5	18.0
1:11–50	1.4	15.5	5.2	16.0	1.9	14.8
1:51–250	0.6	13.0	1.0	17.7	0.6	14.0
1:251–1,000	0.2	11.4	1.0	8.4	0.2	9.9
1:1,001–5,000	0.0	8.9	0.0	4.2	0.2	9.1
1:5,001–25,000	0.0	8.1	0.0	0.8	0.0	3.3
1:25,001–100,000	0.2	3.2	0.0	0.0	0.0	1.6
1:100,001–500,000	0.0	3.2	0.0	0.0	0.0	1.6

These figures do not demonstrate, however, the extent of increase or decrease which occurred in the various samples. Table 3 sets forth the results of calculations of the ratio between the original and final counts. In the determination of the ratio of decrease, the final count was taken as a basis, and as designated

in the upper portion of the first column as 1. In other words if the original count of a sample were 10,000 and the final count were 1000 then the ratio of *decrease* would be 10:1. The opposite calculation was made for the ratio of increase, so that if 1,000 were the original and 10,000 the final count, the ratio of *increase* would be 1:10. This method was considered as giving figures of some significance in showing the extent of increase or decrease in counts. As might be expected the largest percentage of the samples showed increases or decreases within the range of 1:10 or 10:1 respectively. In the case of the unsalted butter the greater tendency was toward larger increases as would be anticipated, especially of mold and bacterial counts. A study of

TABLE 4

Effect of salt content on change in count during storage of butter

SALT CONTENT	TOTAL SAMPLES	DISTRIBUTION OF SAMPLES ACCORDING TO CHANGE IN								
		Mold count			Yeast count			Bacterial count		
		In-crease	No change	De-crease	In-crease	No change	De-crease	In-crease	No change	De-crease
per cent		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0.0	62	85.4	4.0	10 6	71.4	3.4	25.2	72.1	0.0	27.9
0.4–0.95	123	21.0	16.1	62 9	38.7	1 6	59 7	17.7	0.0	82.3
1.0–1.9	207	15.9	13.6	70.5	33.3	4.9	61.8	27.5	0.5	72.0
2.0–3.4	213	29.0	10.7	60.3	29.1	6.6	64.3	26.3	1.9	71.8

the data did not indicate that there was any consistent relationship between low counts and a high ratio of increase or high counts and high ratio of decrease. The increase or decrease apparently was more a function of the types of organisms present in the sample rather than the numbers of them.

Inasmuch as there exists such a marked difference between the changes in salted and unsalted butter, a comparison was made between the changes in samples of different salt content. Table 4 gives the results of such a comparison. It will be noted that there is a sharp line of demarkation between the unsalted and salted samples even when less than 1.0 per cent of salt was present. It is interesting to observe that there was no greater tendency for the more highly salted samples to decrease in count than for those of

lower salt content. This may be explained by the fact that the majority of organisms checked by salt are inhibited by the presence of ralatively small amounts of it. This is especially true in the case of the molds where *Oospora lactis* is the principal type encountered on plates poured from butter and one of the types most susceptible to salt concentration. The data were also studied from the standpoint of the concentration of salt in the brine but the results were practically identical with those presented in table 4. There was a slight indication that the more concentrated brines were somewhat more inhibiting but not strikingly so.

The data in general are interesting and suggestive. The tendency for microörganisms in butter to be checked in their growth by the presence of salt has often been noted. The development of microörganisms in unsalted butter is more extensive as might be expected. One does not interpret qualitative changes in butter on the basis of quantitative changes in the flora but the fact that microörganisms are able to show development in some samples of salted butter and more generally in unsalted butter is suggestive that one cannot overlook the quantitative changes entirely. If some organisms can grow, even though they are harmless, and merely constitute the major portion quantitatively, the more noxious types may also be developing to the detriment of the quality of the butter.

SUMMARY

1. Mold, yeast and bacterial counts of 483 samples of commercial salted butter and 123 samples of unsalted butter, before and after storage, are reported.

2. There is a general tendency for salted samples to show decreases in counts during storage.

3. There is an equally significant tendency for unsalted samples to show an increase in count.

4. The ratio of increase is higher in the unsalted than in the salted butter while the opposite is true for the ratio of decrease.

5. The salt content of butter has a decided effect upon quantitative changes in the microflora of butter during storage but this is not in proportion to the amount of salt present.

SIGNIFICANCE OF COLON-AEROGENES GROUP IN ICE CREAM*

I. SURVIVAL OF MEMBERS OF THE ESCHERICHIA-AEROBACTER GROUP TO PASTEURIZING TEMPERATURES IN ICE CREAM

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INTRODUCTION

The sanitary significance of particular groups of bacteria in food products is of peculiar interest. This is especially true with the Escherichia-Aerobacter group for several reasons. In case of ice cream considerable importance is attached by some to the presence of this group. In this connection several questions present themselves. What are the possibilities of this group gaining entrance into ice cream? Does the presence of this group in retail ice cream indicate inefficient pasteurization or contamination after pasteurization? Is the health officer justified in condemning ice cream which contains members of the Escherichia-Aerobacter group? What is the possibility of members of this group surviving the pasteurizing temperature now in use?

It was to gain specific information on the last named question and general information on some of the other questions raised that this work was undertaken.

The scientific literature contains numerous papers on the bacteriology of ice cream, but very few of these have special reference to the Escherichia-Aerobacter group of organisms. Buchan (3) in 1910 made a complete bacteriological study of 40 small ice cream plants in England. Out of 66 cultures isolated and identified 47 belonged to the Escherichia-Aerobacter group. Isolation was accomplished by the use of bile salt lactose agar. The

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author presented a bacteriological standard for ice cream which limited members of the Escherichia-Aerobacter group to 0.1 cc. of ice cream as measured by the production of acid and gas in bile salt glucose broth. One of the more recent papers on this subject is that of Weinzirl and Harris (16). Three methods for determining the sanitary quality of ice cream were studied and compared. These were the total count, the Escherichia-Aerobacter count and the anaerobic spore test. The authors concluded that the Escherichia-Aerobacter count is of value in controlling the efficiency of pasteurization and that it also indicates insanitary conditions. The fact that it does not distinguish between initial contamination and subsequent multiplication was cited as a distinct disadvantage.

This brief summary indicates the need of further contributions to this field of research. The greatest need seems to be for a standard method of interpreting the significance of members of this group when found in ice cream. However, it is evident that this problem when applied to ice cream presents difficulties not present when applied to milk. The numerous differences between these two dairy products demand more investigation before definite conclusions may be reached. Very little work has been done to indicate those factors which may or may not affect the viability of members of this group in ice cream. This phase alone should be of sufficient value to attract further attention.

PROCEDURE

To test the ability of the Escherichia-Aerobacter group to survive pasteurizing temperatures in ice cream 44 cultures were subjected to three different temperatures. Three cultures of *Aerobacter aerogenes* were isolated from water, 2 from soil, and 2 laboratory stock cultures were used. A total of 33 cultures of *Escherichia coli* were tested. Of this number 13 were isolated from water, 11 were of avian origin, 3 of bovine, 1 of equine, 2 of human, 1 of porcine, 1 of monkey and 1 a laboratory stock culture of unknown origin. In addition to these, 4 lactose-fermenting organisms isolated from water but not definitely classified were also used.

Dolloff's synthetic medium, (6) standard agar and lactose broth to which had been added 1 per cent Andrade's indicator were used. The ice cream mix used in the pasteurizing experiments contained 12 per cent butter fat, 14 per cent sucrose, and 37 per cent total solids. The mix was obtained directly from the holding tank and had been pasteurized previously at 62.8°C. (145°F.). Ten cubic centimeters of this mix were then distributed in pyrex test tubes and heated for 30 minutes in flowing steam for two successive days. The tubes were tested for sterility by seeding into lactose broth.

An experiment was carried out according to the following procedure. The culture to be tested was grown in Dolloff's medium for 24 hours at 37°C. Ten cubic centimeters of sterile ice cream mix were then inoculated with 0.1 cc. of this liquid culture which contained from ten thousand to fifty thousand bacteria as determined by the plate method. The culture was thoroughly stirred into the mix after which the tube was incubated for 2 hours at 37°C. At the end of this time one loop-full (4 mm.) of the material always produced abundant growth and gas in lactose-broth at the end of 24 hours when incubated at 37°C.

At the end of the 2 hours incubation period the inoculated tubes of ice cream mix were placed in an electrically heated and controlled De Khotinsky type water bath which permitted the accurate regulation of the bath temperature to within 0.1°C. After adjusting the bath to the desired temperature, the tube was immersed to within $\frac{1}{2}$ inch of the top. A similar tube of ice cream mix containing a calibrated thermometer was placed along side of the seeded tube. The 30 minute period of heating which was used for all the tests was not recorded until the temperature of the material in the tube containing the thermometer had reached the desired temperature. The temperatures used were 60°C. (140°F.), 62.8°C. (145°F.), 65.6°C. (150°F.), 68.3°C. (155°F.).

Immediately after the 30 minute heating period the tubes were immersed in cold water and 0.1 cc. of the heated mix seeded into a lactose broth fermentation tube. This was incubated at 37°C. for 48 hours and growth recorded. Any fermentation tube showing 5 per cent gas and sufficient acid to change Andrade's indica-

tor at the end of this period was recorded as positive, indicating a survival of the culture at this temperature. Our tests showed that while this method was a more delicate means of indicating the survival of the bacteria to the different degrees of heat used

TABLE 1

The effect on viability of heating members of the Escherichia-Aerobacter group in ice cream mix and in skim milk at a temperature of 60°C. (140°F.) for 30 minutes. Result of four trials

CULTURE NUMBER	ICE CREAM TRIAL NUMBER				SKIM MILK TRIAL NUMBER				CULTURE NUMBER	ICE CREAM TRIAL NUMBER				SKIM MILK TRIAL NUMBER			
	1	2	3	4	1	2	3	4		1	2	3	4	1	2	3	4
2*W	+	−	+	+	−	−	−	−	54A	−	+	−	+	+	+	−	+
3**W	o	o	−	−	−	−	−	−	55A	+	−	−	−	+	−	−	+
7W	o	o	−	−	−	−	−	−	56A	−	−	−	+	+	−	−	+
14*W	o	o	−	−	−	−	−	−	57A	+	−	−	−	−	−	−	−
18W	−	+	+	+	+	+	−	+	58A	−	+	−	+	+	−	−	+
19W	+	+	+	+	−	−	−	−	59A	+	+	−	+	−	−	−	+
29*X	+	−	−	−	−	−	−	−	60A	+	+	−	+	−	−	−	+
30X	−	−	+	+	−	−	−	−	100W	+	−	−	+	−	−	−	+
31*X	−	−	+	+	−	−	−	−	101W	+	+	−	+	−	+	−	+
34**W	−	−	−	−	−	−	−	−	103W	+	+	−	+	+	+	−	+
35**W	−	−	−	−	−	−	−	−	104B	+	+	−	+	+	−	+	+
37**W	−	−	−	−	−	−	−	−	105B	−	+	−	−	−	−	−	−
38W	+	+	+	+	−	+	+	+	107A	−	−	o	−	−	−	−	−
39W	+	+	−	+	−	−	+	−	108E	−	+	o	+	+	−	−	+
40W	+	−	+	−	−	−	−	−	109W	+	−	o	−	+	−	+	−
45W	−	−	+	+	−	−	−	−	110M	−	−	o	−	−	−	−	−
46*W	−	−	+	+	−	−	−	−	112H	−	−	o	−	−	−	−	−
47W	−	−	−	+	−	−	−	+	113H	+	−	o	+	+	+	−	+
49A	+	+	−	+	−	+	+	−	114P	+	−	o	−	−	−	−	−
50A	+	−	−	−	−	−	−	−	116B	−	+	o	+	+	−	+	+
51A	−	−	−	−	−	−	+	−	120*S	+	−	o	+	+	+	−	+
53A	−	+	−	+	+	−	−	+	121*S	+	−	o	−	−	−	−	−

(+) = growth; (−) = no growth; (°) = not determined; (**) = unidentified; (*) = Aerobacter aerogenes; all others *Escherichia coli*. W = water; X = origin unknown; A = avian; B = bovine; E = equine; M = monkey; H = human; P = porcine; S = soil.

than the plate method, yet there was sufficient variation to render the results questionable without several trials. In view of this fact, the entire mix in the tube was incubated at 37° for 48 hours and held at room temperature for 48 hours longer and re-

tested as a check on the lactose fermentation tube method. A negative test was recorded, therefore, only when the lactose fermentation tube failed to show positive and no bacteria were demonstrable in the original mix which had been heated in the tube. This procedure was adopted in view of the work of Ayers and Johnson (2) on "majority" and "absolute" thermal death points of bacteria in milk.

The first temperature used was 60°C. In this experiment the cultures were tested in sterile skim milk as well as in sterile ice cream for comparison. In view of previous work which had

TABLE 2

Showing variability of different cultures when heated at 62.8°C. (145°F.) for 30 minutes in ice cream. Results of four trials

CULTURE NUMBER	NUMBER OF HEATING			
	1	2	3	4
18	+	+	−	+
38	+	+	−	−
39	+	+	−	−
40	+	+	−	−
49	+	−	+	−
60	+	+	+	−
103	−	+	−	−
104	+	+	+	−
107	+	+	−	−
113	+	+	−	−

shown considerable variation when the same cultures were heated several times at the same temperature, the cultures were heated 4 times under identical conditions to test their degree of variability. The results are given in detail in table 1.

On the first trial of the 41 cultures tested, 21 or 51.2 per cent of them survived. Sixteen or 39.0 per cent out of the 41 cultures survived the second trial, while in the third trial only 25 cultures were tested 9, or 36.0 per cent of which survived and on the fourth trial 25, or 57.0 per cent out of the 44 cultures survived.

There were two cultures which appeared to be very resistant to heating in ice cream since they survived all the heatings. Nine

cultures survived 3 heatings in ice cream while the rest were variable. Eleven of the cultures were very sensitive to heat and were killed in each instance.

Skim milk presents quite a different picture. Twenty-two cultures failed to survive any of the heatings. There were none that survived all the heatings; 8 which survived 3 trials while the rest were variable. These data indicate the protective action of ice cream.

TABLE 3

Recapitulation of results secured by heating at temperatures of 60°C., 62.8°C. and 65.5°C. members of the Escherichia-Aerobacter group four times in ice cream

TRIAL NUMBER	TOTAL NUMBER OF CULTURES HEATED	NUMBER KILLED	NUMBER SURVIVING	PER CENT SURVIVING
60°C. (140°F.) for 30 minutes				
1	41	20	21	51.2
2	41	25	16	39 0
3	25	16	9	36 0
4	44	19	25	57.0
62.8°C. (145°F.) for 30 minutes				
1	44	34	10	22 7
2	44	41	3	6 8
3	44	43	1	2.2
4	44	34	10	22 7
65.5°C. (150°F.) for 30 minutes				
1	40	40	0	0
2	41	40	1	2.4
3	41	40	1	2 4
4	44	40	4	9.1

The two most resistant of the cultures were *Escherichia coli* isolated from water. While the number of cultures of *Aerobacter aerogenes* as compared to *Escherichia coli* is too small to draw definite conclusions, yet it would appear from these data that there was little difference in the heat resistance of the two groups.

The temperature was then raised to 62.8°C. for 30 minutes and the experiment repeated with the same cultures using ice

cream and skim milk. Not a single culture survived any of the 4 heatings at this temperature in skim milk. The results were different in ice cream, however, as shown in table 2. In this table only those cultures surviving at this temperature are listed, all the others having been killed. The results of this experiment are given in table 2.

A study of this table reveals that with one exception (culture 107) the cultures surviving at 62.8°C. were likewise resistant to 60°C. However, the number surviving was greatly reduced at the higher temperature since only 10 showed growth at 62.8°C. while 33 survived one or more heatings at 60°C.

Considerable variation in the resistance to heat of different cultures is likewise exhibited in the different trials. In the first trial all survived except one while in the fourth trial all were killed except one.

Since a fairly large percentage of the cultures survived at temperature of 62.8°C. for 30 minutes in ice cream, the temperature was increased to 65.5°C. and the experiment repeated. The results of this experiment are given in table 3. A summary of the results obtained at 62.8°C. and 60°C. are also given for comparison.

Cultures number 18, 29, 38, 39 and 50 survived 65.5°C. Culture 38 survived 2 out of the 4 trials while all the rest survived only 1 out of the 4 trials.

The experiment was repeated again with ice cream. This time the temperature was raised to 68.3°C. and held for 30 minutes. All the cultures were killed.

INFLUENCE OF SEPARATE CONSTITUENTS OF ICE CREAM MIX UPON THERMAL DEATH POINT OF COLON-AEROGENES ORGANISMS

Since the results showed that ice cream mix had a greater protective action than skim milk, further experiments were carried out using the same procedure to determine the effect of each ingredient separately.

In conducting these experiments an attempt was made to study these materials in the same concentration in which they occur normally in the ice cream mix. As the formulae used by the ice cream manufacturers are varied according to the demands

of the consumers, each ingredient was made up in concentrations which would include those used in the various formulae available.

Emulsions of fat were made from 40 per cent cream as a base. In order to avoid changing the solids not fat content, weighed amounts of cream were added to weighed amounts of water. The percentages used were 8 (pH = 6.86), 10, 12, 14, and 16 (each having a pH of 6.94) per cent respectively of butter fat by weight. This material was distributed in 10 cc. amounts in Pyrex tubes and autoclaved for 15 minutes at 10 pounds pressure and checked for sterility. The rest of the procedure was the same as

TABLE 4

The effect on the viability of members of the Escherichia-Aerobacter group when heated in various water suspensions of skim milk powder at a temperature of 60°C. (140°F.) for 30 minutes

CULTURE NUMBER	1 PER CENT pH 6.86		2 PER CENT pH 6.86		4 PER CENT pH 6.70	
	Acid	Gas	Acid	Gas	Acid	Gas
50	—	—	—	—	—	—
51	—	—	—	—	—	—
53	—	—	—	—	—	—
54	—	—	—	—	—	—
55	—	—	—	—	+	+
56	+	+	—	—	—	—
57	—	—	—	—	—	—
58	+	+	—	—	—	—
59	+	+	—	—	—	—

that previously described for the ice cream mix. The temperature used was 60°C. for a period of 30 minutes. Since all the results were consistently negative, they have not been tabulated.

Skim milk powder. Water suspensions of it were prepared in 1, 2, and 4 per cent concentrations. Sterilization was accomplished by successive heatings in flowing steam. The 9 cultures used had previously survived a temperature of 60°C. in skim milk. The results are given in table 4.

The experiments were repeated at a temperature of 62.8°C. for 30 minutes with consistently negative results so they have not been tabulated.

Sucrose was made up in 10, 12 and 14 per cent solutions and tubed in 10 cc. quantities, sterilized at 10 pounds pressure for 15 minutes. The cultures used in this experiment were the same

TABLE 5

The effect on the viability of members of the Escherichia-Aerobacter group when heated in various concentrations of sucrose at a temperature of 60°C. (140°F.) for 30 minutes

CULTURE NUMBER	10 PER CENT pH 6 69		12 PER CENT pH 6.52		14 PER CENT pH 6.18	
	Acid	Gas	Acid	Gas	Acid	Gas
50	—	—	—	—	—	—
51	—	—	—	—	+	+
53	—	—	—	—	—	—
54	—	—	—	—	+	+
55	+	+	—	—	—	—
56	—	—	—	—	—	—
57	—	—	—	—	—	—
58	+	—	+	—	+	—
59	—	—	—	—	—	—

TABLE 6

The effect of the age of the culture when members of the Escherichia-Aerobacter group are heated in ice cream mix at a temperature of 61.6°C. (143°F.) for 30 minutes

NUMBER	6 HOURS		12 HOURS		24 HOURS	
	Acid	Gas	Acid	Gas	Acid	Gas
18	—	—	—	—	+	+
19	—	—	—	—	+	+
38	—	—	—	—	+	+
39	+	—	+	—	—	—
40	+	—	+	—	+	+
60	+	—	+	—	+	+
101	+	—	—	—	+	+
103	—	—	—	—	—	—
Con	—	—	—	—	—	—

as used in skim milk. Three or 33 per cent survived a temperature of 60°C. for 30 minutes. However, all were killed when the temperature was raised to 62.8°C. and held for 30 minutes. The results at 60°C. are given in table 5.

It will be noted that sucrose shows a slight protective action.

Gelatin was tested from two sources of supply. Emulsions were made in concentrations ranging from 0.2 to 1.0 per cent at 0.10 per cent intervals. The pH of each concentration from each supply was determined and found to vary quite widely. The pH of 0.5 per cent solutions of the samples tested were 5.00, 5.67, 5.84, and 7.12 respectively. Nine of the more resistant strains were heated at 62.8°C. for 30 minutes in these different concentrations. All the tests were consistently negative. For this reason the data have not been presented in tabulated form. The acidity of most of the gelatin tested would preclude the possibility of any of the organisms surviving.

THE EFFECT OF AGE OF THE CULTURE

Eight of the cultures showing the greatest resistance to heat were grown for 6, 12 and 24 hours and at the end of these periods were heated to 61.6°C. (143°F.) for 30 minutes. This temperature was chosen since it was found to be the temperature at which the majority of the above strains were still viable at the end of 30 minutes. The results are given in table 6.

DISCUSSION

The data submitted have provided some interesting facts which should prove useful in interpreting the significance of members of the Escherichia-Aerobacter group in ice cream. The comparison of the effect on viability produced by heating the different cultures in skim milk and in ice cream at the various temperatures for 30 minutes indicated definitely that the latter exhibited a greater protective action.

The testing of the ingredients contributed very little to a solution of the problem as a whole. Of all the substances tested fat was the one from which the greatest protective action was expected. The results of heating of the cultures in fat suspensions showed great variability. However, the organisms were readily killed when heated at a temperature of 60°C. for 30 minutes. The data show no influence of concentration of fat upon viability.

Heating the cultures in skim milk powder showed no correla-

tion between concentration and viability. The cultures surviving a temperature of 60°C. when heated in skim milk powder were the same cultures surviving this temperature when heated in skim milk. Also three of these cultures survived in ice cream mix at the same temperature.

Sucrose showed some protective action since some of the cultures survived at 60°C.

Gelatin was the one ingredient tested which exhibited the least protective effect at the concentration used (0.5 per cent). The lowest temperature adopted, 60°C., was sufficient to kill all cultures heated.

The marked effect exhibited by the age of culture was found to be in accord with previous work of this nature. Thus Robertson (11) found that young cells were more susceptible than older cells to the action of high temperatures. He concluded that the most efficient pasteurization would be obtained if the heat was applied before the bacteria had passed the accelerative stage of growth. Sherman and Albus (12) found that young cells of bacteria are more easily killed by heat and various harmful agents then are the old cells of the same organism. Sherman, Stark and Stark (13) more recently have reviewed the literature in this connection and have given additional data to support this view.

The variations and apparent inconsistencies were not entirely unexpected since other workers have found similar variations. Ayers and Johnson (1) studied the effect on viability of 174 cultures of *Escherichia coli* when heated in milk and found the following variations: At a temperature of 62.8°C., 12 cultures survived the first trial. On retesting they found 4 cultures surviving the second trial, 8 surviving the third trial, 6 surviving the fourth trial, 9 surviving the fifth trial, and no cultures surviving the sixth trial. The litmus milk tube method was employed. The authors concluded that a temperature of 65.5°C. for 30 minutes should destroy members of the Escherichia-Aerobacter group when heated in milk. Tanner and Windsor (15) studied 23 cultures of *Escherichia coli* and found only one strain that repeatedly withstood heating in sealed tubes for 30 minutes at 62.8°C. If it is common for milk to show marked variations, it would not be surprising that the ice cream mix should exhibit even greater variations.

The question of pH undoubtedly is closely linked with viability. According to Cohen (5) mortality is affected by small changes in pH. This factor would be impossible to control except under laboratory conditions. The mix when stored in the holding tank slowly undergoes a decrease in pH. Thus freshly made mix was found to have a pH of 6.8, whereas after 48 hours in the holding tank the pH had often dropped to 5.5. The determination of the significance of this factor would constitute a separate problem in itself.

One of the outstanding characteristics of ice cream mix is its viscosity. It seems reasonable to assume that this property might have some influence on the protective action exhibited by the mix. Extensive work by Joslyn (8) on sirups showed the relationship between viscosity and heat penetration. He found that an increase in viscosity resulted in a decrease in the rate of heat penetration and that the addition of acid reduces the retardation of heat penetration. This factor which has little significance under laboratory conditions, might be of importance in the commercial pasteurization process.

The fact that ice cream mix does possess considerable protective action for members of the Escherichia-Aerobacter group is significant. Thus it is reasonable to assume that this protection might be accorded to other groups of bacteria. If this assumption is correct, it is evident that a temperature of 62.8°C. cannot effectively pasteurize ice cream mix.

SIGNIFICANCE OF ESCHERICHIA-AEROBACTER IN ICE CREAM

Inasmuch as ice cream is a dairy product and the conditions under which dairy products are produced precluded the possibility of entirely eliminating members of this group, it would seem that their presence in ice cream is to be expected to a certain extent. Furthermore, it has been shown that certain strains are able to survive the temperature which is generally used in pasteurizing ice cream mix viz. 62.8°C. for 30 minutes. It would seem, therefore, that qualitative tests would be of little value in this connection. Definite quantitative tests plus a history of the sample being tested would be of value.

It has been pointed out by Mudge (9) and Price (10) that the significance of the colon organism when found in milk is in no way as great as when found in water.

There are some, however, who consider that *Escherichia coli* is a valuable index of pasteurizing efficiency and that it can be used to check up on plant performance.   Jenkins (7) in England studied the flora of pasteurized milk and stated that an effectively pasteurized milk should not contain lactose fermenting bacilli in one cc. and that *Escherichia coli* is a valuable index of the efficiency of the pasteurizing process.   Swenarton (14) likewise believes that a test for *Escherichia coli* content can be used to check up on plant procedure since the charts indicated a definite correlation between coli content and plant procedure.   He found that control charts from milk plants whose milk was high in coli content showed improper heating or irregularity of procedure.

The one other factor of interest, namely, that of contamination after pasteurization is one which should be given due consideration.   Probably the most common source of contamination of this type is from the hands of operators.   The possibility of this source of *Escherichia coli* was recognized by Winslow (17) as early as 1903.   A more recent article by Buice, Sehested and Dienst (4) reported 337 tests made on hands of 251 food handlers. They found 67.7 per cent of the total number showing lactose fermenting aerobes.   By culturing with Koser's sodium citrate medium, 8.4 per cent of the total proved to be of intestinal origin. Close attention to personal hygiene would eliminate this factor as one of importance.

SUMMARY AND CONCLUSIONS

1. The thermal death point of 33 cultures of *Escherichia coli*, 7 cultures of *Aerobacter aerogenes* and 4 lactose-fermenting organisms isolated from water but not definitely classified showed considerable variation when heated in ice cream at temperatures of 60°C. (140°F.), 62.8°C. (145°F.) and 65.5°C. (150°F.) for 30 minutes.

Four determinations were made at a temperature of 60°C. The number surviving expressed in per cent was 51.2, 39.0, 36.0

and 57.0 respectively for each determination. When the temperature was increased to 62.8°C., the number surviving decreased somewhat being 22.7, 6.8, 2.2 and 22.7 per cent respectively for the successive determinations. At a temperature of 65.5°C. the number surviving is still further decreased and was 0.0, 2.4, 2.4 and 9.1 per cent respectively while at a temperature of 68.3°C. none of the cultures tested survived.

These data indicate that the critical temperature for the Escherichia-Aerogenes group in ice cream is about 65.5°C.

2. Ice cream has a greater protective action than skim milk for members of the Escherichia-Aerogenes group when heated at temperatures of 60°C. and 62.8°C. for 30 minutes. At the former temperature in skim milk there were 22 cultures out of the 44 cultures used which failed to survive any of the four heatings as compared to 11 cultures in ice cream. When the temperature was raised to 62.8°C. all the cultures were killed in skim milk in each instance while in ice cream there were certain cultures still surviving.

3. Thermal death point determinations made with the cultures in the different ingredients used in making ice cream, viz. cream, sucrose, milk powder and gelatin failed to show any marked protective action of any one of the ingredients.

4. The susceptibility of bacterial cells to heat is greatly influenced by their age, young cells are more readily killed than are the older ones.

5. The ability of many strains of the Escherichia-Aerogenes group to survive a temperature of 62.8°C. and even 65.5°C. in ice cream should be taken into consideration when using the colon test as an index of the efficiency of pasteurization.

6. From the standpoint of reduction in numbers of the Escherichia-Aerogenes group, pasteurizing the ice cream mix at a temperature of 65.5°C. is more desirable than the lower temperature 62.8°C. which is more commonly used.

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A CASE OF SANDINESS IN PROCESSED CHEESE*

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This brief article is to record the occurrence of "sandiness" in processed cheese. Two samples of sandy processed cheese were examined independently by the writer and by the Wisconsin Dairy and Food Commission chemists, respectively. In the former case, the sample was submitted by the manufacturer for identification of the gritty particles and diagnosis of the defect; in the latter case, the sample was obtained from a retailer after a consumer had complained that the cheese contained broken glass. It is not definitely known, but it is likely that both samples originated from the same source.

The gritty particles were easily visible to the naked eye and were obviously crystalline. The appearance and sensation on chewing was as though quartz sand had been incorporated into the cheese. Figure 1 is a photomicrograph, magnification 24 times, of the gritty particles removed from the cheese.

Some of the gritty particles were removed with the aid of a scalpel, and cleaned by removing adhering cheese and washing successively with water, alcohol and ether. The crystals were soluble in hydrochloric and nitric acids, and disintegrated in sulphuric acid, leaving a fine precipitate (calcium sulphate). Calcium was demonstrated to be present by the oxalate test applied either to a solution obtained by dissolving the residue after ashing some of the particles, or to a solution obtained by dissolving some of the particles in nitric acid, evaporating to dryness and taking up the residue with water. The acid in combination with calcium was found to be an organic acid. The crystal structure of the gritty particles suggested that they consisted of calcium tartrate. Tartaric acid was demonstrated by the re-

ducing action on silver nitrate in ammoniacal solution causing the deposition of a silver mirror in a clean test tube, and by Denige's modification of Mohler's Resorcinol Test.   The conclusion was that the gritty particles were calcium tartrate.   Both laboratories working independently and without knowledge of the other's work on a similar sample, arrived at the same conclusion.   In the case of the sample submitted by the manufacturer it was defi-

Fig 1. Photomicrograph of Calcium Tartrate Crystals Taken from Sandy or Gritty Processed Cheese.   Dark Field Illumination, Magnification 24 ×

nitely ascertained that the processed cheese had been made with the addition of Rochelle Salts as an emulsifying agent.

In an isolated attempt to produce this sandy condition by processing cheese with the addition of 2.0 per cent Rochelle Salts and 0.2 per cent calcium acetate, the sandy condition did not result.   The solubility of calcium tartrate is 0.016 parts by weight

per 100‾parts of water at 15°C.   From this fact, from the known water and calcium content of average cheese, and from the fact that emulsifying agents in processed cheese manufacture are used in amounts ranging from 0.5 to 3.0 per cent, it is quite certain that precipitation of calcium tartrate always occurs in processed cheese when tartrate is used as the so-called emulsifying agent. The question of whether or not the cheese becomes sandy or gritty must then be a question of the size of calcium tartrate crystals, rather than their presence or absence.   A number of conditions likely to affect the size of the calcium tartrate crystals suggest themselves, but have not been subjected to experimental study.

CONCLUSION

In two samples of gritty or sandy processed cheese, the cause of the defect was the presence of calcium tartrate crystals of macroscopic size.

OPTIMUM AMOUNT OF SILAGE IN THE DAIRY RATION FOR MOST ECONOMICAL PRODUCTION*

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PRELIMINARY STATEMENT

Following the introduction of the silo on farms in the eighties a conviction of the great importance of corn silage in the dairy ration has become almost universal. These opinions have been formed and sustained by numerous reports from experiment stations and by practical feeders as well. In more recent years the ensiled product of various other plants has been reported favorably. Moreover the extension of the uses of silage to beef cattle production and to the feeding of sheep and horses has been acclaimed with enthusiasm.

The point of view with which the subject usually has been approached experimentally in dairy cattle feeding has involved an attempt to discover the influence upon milk yield of substituting silage for a portion of the dry roughage of the ration. Under such trials the animals have usually responded by an increased yield and this has furnished the basis for proclaiming silage as the one feed to be desired above all others, without adaptation to any particular farm, area, or region.

During the past few years the Farm Management Department of the Connecticut Agricultural College has observed the cost of corn production in Connecticut to be relatively high compared to hay production, due to the intensive labor and fertilizer requirements of corn. Furthermore, this matter has attracted the attention of the farmers themselves, with the result that many have turned to large Southern varieties in an effort to reduce the acreage devoted to corn and to obtain cheaper silage. In more

recent years corn production for silage has been curtailed to a noticeable extent, and in a few cases abandoned, even on large farms. This situation applies at least in southern New England and probably constitutes a similar problem in other sections as well.

In conjunction with other considerations in the silage feeding trials under way at Storrs since 1921, succeeding variety tests by the Department of Agronomy, the question of the optimum amount of silage for dairy cattle rations under prevailing conditions has continually injected itself into the problem. Only recently has it been possible to attack the problem directly. The preceding trials have successively shown: (a) that large Southern varieties[1] which yield the largest gross tonnage of dry matter are capable of producing the most milk per acre of corn (1); (b) that although such varieties yield silage containing considerably less grain and a lower percentage of dry matter, accompanied by a somewhat larger percentage of fiber per unit of dry matter, yet the dry matter of such corns does not suffer appreciably in feeding value, the result being that the acre dry matter production is a better criterion for measuring the production ability of a given variety of corn than its ability to form ears in an average growing season (2); (c) a cow receiving the standard amount of silage will adjust her total dry matter consumption by ingestion of hay in amount to correspond with the dry matter content of the silage (unpublished data (3)).

In consideration of the change in economic conditions favoring more and better hay production on tillable land, and with an appreciation of the marked advance that has taken place in the feeding of better grain rations to cows during the past few years, the authors felt that it was timely to attack the problem from this new point of view. The question may be propounded: "Does the cow now respond as favorably to silage as she did upon the prevailing rations in use at the time of and for several years following the introduction of the silo?"

[1] The growing season for corn is about 120 days in Connecticut.

HISTORICAL STATEMENT

It does not seem appropriate to review here all of the numerous experiments in which silage has had an important rôle. Only those involving considerations of corn silage and hay which afford sufficient data to permit of a comparison of the total dry matter consumed, the resulting milk yield, and feed costs of the milk, will be mentioned.

Most of the early investigators were concerned directly in finding whether silage could be substituted for hay, without much attention to the proportion of each which would prove most efficient and economical. Due to the usual greater yield of dry matter from an acre of corn, few, until recent years, seem to have considered that corn silage may on some farms and in some areas be more costly than hay.

Hills (4) in 1889, Bartlett (5) in 1889, and Wheeler (7) in 1895 all report greater milk yield and more efficient use of dry matter when corn silage replaced a part of the hay in the ration.

Fairchild and Wilbur (8) at Indiana, Carroll (9) at Utah, and the Arizona Station (10) report an increase in milk production by replacement of a part of the alfalfa hay by silage. Silage is estimated to have a value ranging from 28 to 40 per cent of that of an equal weight of alfalfa, although in two cases no analyses are given.

The results referred to, together with the data of the present trial, are summarized in table 7. Wherever possible the yields have been calculated to 4 per cent milk using the formula of Gaines and Davidson (6) (0.4 × milk + 15 × fat).

OBJECT OF EXPERIMENT

In actual feeding practice upon the dairy farm the relative amounts of corn silage and home grown hay available determine to a large extent the proportions of each in the ration. Under conditions prevailing in Connecticut (limited tillable land on many farms) faith in silage often dictates the production of corn on tillable areas to the exclusion of hay except on permanent hay fields, necessitating the use of much poor hay and the limited feeding thereof.

Studies in recent years by the Farm Management Department at the Connecticut Agricultural College have revealed that Connecticut farmers expend about 80 hours of man labor in growing and ensiling an acre of corn, and that a ton of silage costs the Connecticut dairyman fully half as much to produce and harvest as a ton of hay.

These findings together with results in silage investigations by the Department of Dairy Husbandry, already referred to, have raised the pertinent question as to what proportion of hay and silage in the ration is desirable in the interest of greatest profits.

The experiments referred to uniformly indicate that corn silage when added to a ration tends to increase milk flow. Many practical feeders, although not all, will testify to this fact. Notwithstanding the somewhat overwhelming evidence in this direction some important questions yet remain unanswered, namely: (a) what is the optimum amount of silage for a dairy cow necessary to maintain satisfactory yield; (b) what is the minimum amount of silage capable of furnishing succulence to the ration; (c) what is the feed cost of milk production using varying proportions of silage in the ration and hay ad libitum in each case; (d) may not the improved quality of hay and of grain mixtures have lessened the favorable influence of silage as determined by earlier feeders and investigators?

It is hoped that the present and subsequent experiments will throw light on such questions. This experiment (the eighth in silage investigations) was designed to show whether light or heavy silage in the ration would be, *first*, more efficient and *second*, more economical.

EXPERIMENTAL PROCEDURE

Eight purebred cows, 4 Holsteins and 4 Guernseys, were divided into two groups evenly balanced with respect to breed, body weight, age, milk production, and stage of lactation. The animals were also closely balanced with respect to their body type and ability to consume roughage, factors of much importance in an experiment of this kind.

Table 1 gives data concerning the experimental cows.

The groups, it will be observed, are especially well balanced, perhaps excepting date of conception. At this stage of gestation it can be assumed that there was little influence upon results due to this factor.

One group was fed 36 pounds of silage and the other 18 pounds per cow. The grain was fed in proportion to milk flow and hay allowed ad libitum in two feedings.

Two preliminary periods of 10 days each (January 2-21, inclusive) were provided to allow each animal to be changed over

TABLE 1

Data concerning the animals

NAME	BREED	AGE	CALVED	CONCEIVED	INITIAL WEIGHT
Heavy Silage group					
Delight	Holstein	2⁻ 9-14	8-31-28	11-21-28	1,270
Senora	Holstein	2-10-20	8- 9-28	11-18-28	1,185
Amiable	Guernsey	4- 1-16	10-15-28	12-25-28	1,002
Sagacious....................	Guernsey	5- 4-13	12- 8-28	1-18-29	1,050
Average age and weight		3- 9-16			1,127
Average days from calving..	89				
Light Silage group					
Korndyke....................	Holstein	2- 8-15	8-12-28	1-26-29	1,162
Queen.......................	Holstein	2-10-19	8- 5-28	11-20-28	1,278
Buoyant......................	Guernsey	4- 9-12	12-20-28	2-10-29	1,054
Laureate....................	Guernsey	5- 8-18	11- 6-28	1- 1-29	1,123
Average age and weight.....		4- 0- 8			1,154
Average days from calving..	91				

from herd routine, to become accustomed to the ration and stabilized in weight and production. The experiment proper in addition consisted of ten 10-day periods from January 22 to May 1 inclusive.

Since the roughages and the grain fed to both groups were from the same source daily there was no appreciable opportunity for variation in quality of roughage or grain to affect the experimental results.

Silage feeding

The silage was of the variety *early mastodon*, classed as moderately late maturing, and containing a small proportion of developed ears. Analysis revealed an average dry matter content of 21.90 per cent. The silage was removed from the silo daily with that fed the main herd and was weighed into the manger from the feed truck morning and evening. All silage refused was weighed and recorded. The silage was sampled at the middle of each 10-day period and air dried immediately with the aid of a fan before submitting for analysis.[2]

Hay feeding

The hay was fed twice daily, immediately following the consumption of silage and grain, in such amounts as the individual cows preferred. Any portion not eaten by an animal was weighed and recorded.

Since it was planned to make the results of this experiment applicable to average Connecticut herds it seemed advisable to use average hay. It was decided that mixed hay containing some clover should be used. Because of the extremely rainy season of 1928 and the difficulty experienced in curing, the hay, which was purchased from a Connecticut farm, did not prove to be of very good quality. It was cut fairly early and contained not over 15 per cent clover, but many bales were musty, making careful sorting necessary. A characteristic flake was removed from each bale and composited for the 10-day sample. Later a composite was removed from each 10-day sample for an experimental sample. The sample covering the first half of the experimental period was inadvertently destroyed (by an employee) so the analyses given for hay cover only the last half of the period. Observation indicated that the sample as analyzed probably was somewhat superior to that fed during the first half of the period.

Grain feeding

The grain fed was a stock mixed open formula ration with a guarantee of 20 per cent protein prepared by the Eastern States Farmers' Exchange. The analysis, shown in table 3, was made from a composite sample from every fifth bag. The ingredient formula follows:

pounds

Corn Gluten Feed. 380
Choice Yellow Hominy 340
Linseed Oil Meal O. P. 320
Pure Ground Oats (No. 2—38 pounds clipped)....................260
Standard Wheat Bran..200
Choice Cottonseed Meal 140
Corn Distillers Dried Grains 100
Standard Wheat Middlings 100
Molasses 100
Steamed Bone Meal 20
Calcium Carbonate 20
Salt. 20

The grain was fed approximately at the ratio of 1 pound to each 3 pounds of milk produced, with allowance for differences in test, but was varied individually, however, in an attempt to hold body weight uniform. The grain was fed twice daily on the silage.

Management of cows

The cows were kept in stanchions and turned out an hour or two daily for exercise when the weather was suitable. Water was before them at the stanchions. Since the grain ration contained 1 per cent salt no additional salt was fed. Wooden manger partitions were installed to prevent the animals from thieving. Sawdust was used for bedding so there was no opportunity to consume straw and vitiate the dry matter intake. Each animal was weighed daily between 8 and 9 A.M. The weights as presented are an average of the weights the day before, the day of, and the day following the date given in table 5. The cows were fed silage and grain at 5 A.M. and at 4 P.M. After these were eaten hay was fed. The cows were hand milked while consuming silage and grain.

Presentation of data

There was a tendency on the part of 6 of the 8 cows to consume more hay during the preliminary period than they could continue

TABLE 2

Group feed records

	HEAVY SILAGE GROUP			LIGHT SILAGE GROUP		
	Silage	Hay	Grain	Silage	Hay	Grain
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
First preliminary period....	1,434.3	455.6	380 00	720	578.1	377.6
Second preliminary period..	1,438.5	447.5	361 8	720	535.1	361.8
First experimental period...	1,440.0	440.3	336.1	720	527.1	342.3
Second experimental period.	1,411.4	436.2	316.1	720	539.4	323.8
Third experimental period..	1,431.5	374.3	309.1	720	540.8	311.5
Fourth experimental period.	1,440.0	365.4	301.1	720	512.6	301.8
Fifth experimental period...	1,438.4	361.4	293.5	720	512.1	282 6
Sixth experimental period...	1,440.0	371.6	296 0	720	471.1	275.5
Seventh experimental period.	1,436.5	370.1	292.7	720	484.5	254.1
Eighth experimental period.	1,428.0	350.3	287.3	720	473.5	267.9
Ninth experimental period..	1,468.5	337.9	286.0	720	475 6	279.1
Tenth experimental period..	1,473.9	341.5	276.0	720	483.0	277.5
Total (100 days)............	14,408.2	3,749 0	2,993.9	7,200.0	5,019.7	2,916.1
Average per day, experimental period..........	36.02	9.37	7.48	18 00	12.55	7.29

TABLE 3

Average analyses of feeds in per cent

	DRY MATTER	MOIS-TURE	ASH	PROTEIN	FIBER	N-FREE EXTRACT	FAT
Silage....................	21.90	78.10	1.42	1.73	6.60	11.59	0.56
Hay......................	92.84	7.16	5.83	8.25	.36.24	40 85	1.67
Grain....................	91.35	8.65	6.50	21.13	7.71	51.55	4.46

to eat, evidence of which is found in the records of feed consumption by 10-day periods in table 2.

It will be noted also that the grain feeding was unnecessarily high at the start, especially for the lower producers, necessitating rather sharp reductions during the first few periods.

TABLE 4

Group production records

	HEAVY SILAGE GROUP			LIGHT SILAGE GROUP		
	Milk	Fat	4 per cent milk	Milk	Fat	4 per cent milk
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
First preliminary period....	1,223.7	50 866	1,252.4	1,112.8	46.881	1,148.4
Second preliminary period..	1,174.4	47.130	1,174.8	1,038.7	48.977	1,150.0
First experimental period...	1,122.5	43.912	1,107.6	985.1	46.163	1,086 8
Second experimental period.	1,054.4	36.397	967.6	938.7	42 047	1,006.4
Third experimental period..	993.6	37.926	956.4	883.2	40.803	965.6
Fourth experimental period.	953.5	34.431	898.4	837.5	40.299	939 6
Fifth experimental period ..	925.3	35.388	900 8	824.4	38.416	906.0
Sixth experimental period...	927 2	36.258	914.8	790.0	35.639	850.8
Seventh experimental period.	857.6	34.642	862.8	706 9	31 389	753 6
Eighth experimental period.	801.5	31.151	788 0	722.7	32.829	781 6
Ninth experimental period..	738 3	29 266	734.4	681.1	31.018	737.6
Tenth experimental period .	678.6	28.709	702.0	658.1	29.663	708.0
Total (100 days)............	9,052 5	348.080	8,832.8	8,027.7	368.266	8,736 0
Average per day, experimental period	22 63		22.08	20 07		21.84

TABLE 5

Average body weights of groups

	FIRST PRELIMINARY PERIOD		SECOND PRELIMINARY PERIOD		FIRST EXPERIMENTAL PERIOD		SECOND EXPERIMENTAL PERIOD	
Date....	1–3	1–8	1–13	1–18	1–23	1–28	2–2	2–7
Heavy Silage group.......	1,135	1,135	1,137	1,139	1,140	1,140	1,133	1,129
Light Silage group........	1,154	1,148	1,139	1,143	1,134	1,128	1,127	1,124

	THIRD EXPERIMENTAL PERIOD		FOURTH EXPERIMENTAL PERIOD		FIFTH EXPERIMENTAL PERIOD		SIXTH EXPERIMENTAL PERIOD	
Date.....................	2–12	2–17	2–22	2–27	3–4	3–9	3–14	3–19
Heavy Silage group.......	1,120	1,114	1,103	1,105	1,105	1,103	1,110	1,114
Light Silage group........	1,127	1,130	1,121	1,121	1,113	1,108	1,114	1,101

	SEVENTH EXPERIMENTAL PERIOD		EIGHTH EXPERIMENTAL PERIOD		NINTH EXPERIMENTAL PERIOD		TENTH EXPERIMENTAL PERIOD	
Date.....................	3–24	3–29	4–3	4–8	4–13	4–18	4–23	4–28
Heavy Silage group........	1,113	1,118	1,117	1,117	1,109	1,118	1,118	1,119
Light Silage group........	1,086	1,097	1,092	1,085	1,087	1,094	1,093	1,090

TABLE 6

Group consumption of dry matter in pounds

PERIOD	HEAVY SILAGE GROUP				LIGHT SILAGE GROUP			
	Silage	Hay	Grain	Total	Silage	Hay	Grain	Total
First preliminary	333.47	422.98	347.13	1,103.58	167.40	536.71	344.94	1,049.05
Second preliminary	349.99	415.46	330.50	1,095.95	175.18	496.79	330.50	1,002.47
First experimental	325.44	408.77	307.03	1,041.24	162.72	489.36	312.69	964.77
Second experimental	306.98	404.97	288.76	1,000.71	156.60	500.78	295.79	953.17
Third experimental	314.93	347.50	282.36	944.79	158.40	502.08	284.56	945.04
Fourth experimental	338.83	339.24	275.05	953.12	169.42	475.90	275.69	921.01
Fifth experimental	322.63	335.52	268.11	926.26	161.50	475.43	258.16	895.09
Sixth experimental	325.15	344.99	270.40	940.54	162.58	437.47	251.67	861.72
Seventh experimental	312.01	343.60	267.38	922.99	156.38	449.81	232.12	838.31
Eighth experimental	305.74	325.22	262.45	893.41	154.15	439.60	244.73	838.48
Ninth experimental	310.29	313.71	261.26	885.26	152.14	441.55	254.96	848.65
Tenth experimental	292.57	317.05	252.13	861.75	142.92	448.42	253.50	844.84
Total experimental (100 days)	3,154.57	3,480.57	2,734.93	9,370.07	1,576.81	4,660.40	2,663.87	8,901.08

Table 3 presents the chemical analyses of feeds used during the experiment. The silage analyses are an average of the 10 samples for the experiment proper; however for purposes of computation the several individual analyses were used for their respective periods.

Table 4 gives the group production of milk and fat by periods, also the 4 per cent fat corrected milk. Gaines and Davidson (6) submit abundant data justifying the practice of correcting milk production to 4 per cent fat basis for purposes of comparison.

Average group weights are given in table 5.

The Heavy Silage group lost an average of 21 pounds per animal while the Light Silage group lost an average of 44 pounds from the first 3 days of the first experimental period to the last 3 days of the last experimental period.

From the analyses given for hay and grain, and from silage analyses for the respective periods, and also from the data on food consumption in Table 2 the dry matter consumption for the two groups was computed. This is presented in table 6 for the several periods.

<center>DISCUSSION</center>

Reference to table 2 will show that both groups consumed more hay for the first two experimental periods than they were capable of consuming indefinitely at the level of silage intake. The break in hay consumption of the Light Silage group in the sixth experimental period was due to the fact that Queen went off feed. She was a cow with considerable total capacity for roughage consumption, similar in this respect to Delight in the other group, and ate more over a period of time than proved to be advantageous to herself. This parallels and compensates the action of Delight who was more than any other responsible for the decline in hay consumption of the Heavy Silage group during the third experimental period. These examples illustrate the need of a long experimental period and also illustrate the opportunity for a residual effect to be carried over into the experimental period if the reversal type of experiment is employed. It will be noticed that the Heavy Silage group continued to decline in hay con-

sumption to the ninth experimental period, influenced in part perceivably by advancing lactation.

It will be noted from table 5 that the two groups were evenly balanced in weight at the beginning of the preliminary period and had an approximately equal weight at the beginning of the experimental period proper. There are three changes in the weights which deserve explanation; first, the Light Silage group decreased in weight during the preliminary periods probably due to loss of fill of the intestinal tract; second, the loss in weight of the Heavy Silage group occurred in the third period when Delight over-ate followed by a decrease in grain consumption temporarily; and, third, decline in the weight of the Light Silage group which occurred March 19 was due to an udder injury to Korndyke causing a decline in milk production and necessitating a temporary cut in grain feeding. At this same time Queen, in the same group, over-ate, adding to the decline in weight.

An analysis of the production of 4 per cent milk and the consumption of grain for the two groups shows that the Heavy Silage group produced 2.951 pounds of milk and the Light Silage group 2.996 pounds of milk per pound of grain fed, or that the Light Silage group received slightly less grain per pound of milk produced. Since the two groups were carefully balanced in all respects and were fed almost an identical ratio of grain per pound of milk produced it is reasonable to assume that any differences in efficiency and economy of production are directly due to the relative proportions of the dry matter coming from silage and hay respectively. Reference to table 6 shows that the Light Silage group failed by 397.9 pounds of dry matter to eat sufficient additional hay to compensate for the lower silage allowance. This doubtless accounts for the greater loss of weight of this group, 44 pounds per animal against 21 for the other group.

With respect to milk production per pound of dry matter consumed the Light Silage group produced 0.98 of a pound of milk and the Heavy Silage group 0.94 of a pound of milk for each pound of dry matter in the ration. Expressing this in another way the Light Silage group consumed 102 pounds of dry matter to 106 pounds by the Heavy Silage group for each 100 pounds of

4 per cent milk yielded. This seemingly greater efficiency of the Light Silage group has the greater loss of body weight to be charged against it, which for want of an accurate correction factor must be assumed to balance the two groups. Both differ-

TABLE 7

Comparison of work at several stations showing the pounds of milk* produced per pound of dry matter fed and the per cent of dry matter provided by the hay and silage

STATION	PERIOD	4 PER CENT MILK PER POUND DRY MATTER	PER CENT DRY MATTER	
			Hay	Silage
Connecticut (Storrs)	Heavy Silage	0.94	37.1	33.7
	Light Silage	0.98	52.4	17.8
Maine	No Silage	0.94	73.6	
	Heavy Silage	0.90	50.6	26.0
	No Silage	0.86	74.8	
Vermont	No Silage	0.74	70.1	
	Silage	0.81	40.2	27.2
Geneva.	No Silage	0.67	72.7	
	Light Silage	0.72	51.0	20.3
	Heavy Silage	0.77	27.6	41.2
Geneva.	Heavy Silage	0.92	27.6	42.8
	Light Silage	0.91	46.1	24.0
Geneva.	Light Silage	0.94	49.7	22.8
	Heavy Silage	1.00	26.0	45.2
Purdue	Heavy Silage	0.94	35.9	39.1
	No Silage	0.92	75.0	

* Computed by the authors to a 4 per cent fat basis.

ences, however, are well within the range of experimental error, or chance.

Table 7 presents a comparison of results of other experiments with these under discussion.

In evaluating the data in table 7 it should be kept in mind that the number of animals in each group is small, but it seems signif-

icant that all of the older data indicates more efficient production with the larger amounts of silage. Furthermore, silage feeding has resulted in a more efficient production in respect to the dry matter of the ration than when no silage was fed. But from the data in these experiments it is impossible to determine the changes in weight of the groups under comparison.
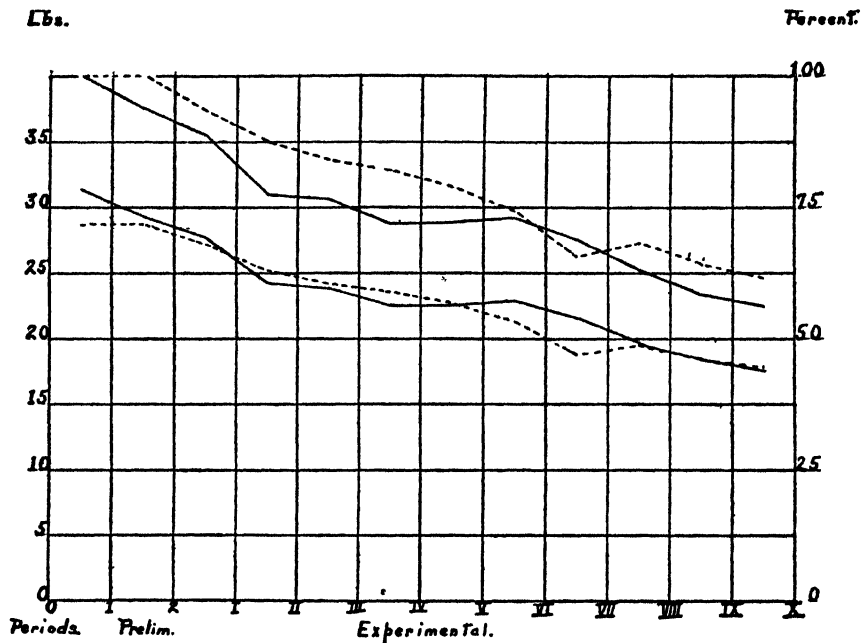
FIG. 1. DECLINE OF 4 PER CENT MILK

The upper graph is expressed as the percentage decline taking the first month as 100, while the lower graph is expressed in pounds.

Heavy Silage group ——————.
Light Silage group ----------.

The Storrs data, which is in disagreement, may or may not be significant. The problem requires further experiment.

In studying all of the data it is found that there is after all comparatively little difference in efficiency of rations with large or small amounts of silage, and that the ultimate feeding practice will depend therefore upon whether or not silage in quantity

stimulates greater milk flow or appetite, and the relative cost of dry matter from silage and hay.

Turner (11) in studying the rate of decline of milk production found that on an average the monthly milk yield is 93.95 per cent of that of the preceding month. The Light Silage group averaged 95.4 per cent of the previous months production while the Heavy Silage group averaged 95.0 per cent. In this respect the groups seem to have been well balanced and the yield well maintained. Turner found that food consumption averaged 97.98 per cent of that of the previous month. The Light Silage group averaged 98.2 per cent while the Heavy Silage group averaged 97.7 per cent. Likewise the results were similar in this respect. Figure 1 illustrates the decline of milk production in pounds and in per cent.

The Farm Management Department at the Connecticut Agricultural College has estimated from farm accounts the cost of production of silage at $9.00 per ton and of hay at $16.00 per ton (unpublished data). With grain at cost ($52.35 laid down at the barn in the winter of 1928–29 wholesale) and roughage at the above prices, the feed cost of producing 100 pounds of 4 per cent milk was $1.95 for the Heavy Silage group and $1.70 for the Light Silage group. This difference in economy of production resulted in a 14 per cent greater income above feed cost for the Light Silage group despite the slightly greater milk yield of the Heavy Silage group.

By calculation it is found that the prices of hay and silage would have to be established at $25.00 and $5.00 respectively, a relation which would scarcely be possible, to make the feed cost approximately equal for the two groups. At these assumed prices for roughage the feed cost per 100 pounds of milk of the Heavy and Light Silage groups would be $1.82 and $1.80 respectively.

Adjusting the price of grain to correspond to different geographical areas will not materially alter the relationship. It would seem from this data that the hay is worth about five times as much as the silage. Wheeler (Geneva), from his experiments, stated that with hay at $10.00 per ton the silage was worth $2.62. At this ratio with hay valued at $16.00 per ton the silage would

have to be produced at $4.19 per ton to be on a par in cost. The silage in this instance (Wheeler's experiment) contained 26.34 per cent dry matter.

SUMMARY

Two groups of milk cows were placed on experiment for a preliminary period of 20 days and an experimental period of 100 days. One group received 36 pounds of corn silage daily while the other received but 18 pounds. Both were fed approximately 1 pound of grain for each 3 pounds of 4 per cent milk produced and mixed hay, largely timothy, ad libitum.

Heavy silage feeding resulted in slightly greater dry matter consumption and in slightly greater milk production. The Light Silage group produced slightly more milk per unit of dry matter, but on the other hand lost more weight. However the amount of weight lost in either case is no more than would be expected for cows in the first half of their lactation when producing efficiently.

With hay at $16.00, silage at $9.00, and grain at $52.35 per ton the feed cost of 100 pounds of milk was $1.95 for the Heavy Silage group and $1.70 for the Light Silage group. In this trial the income over feed cost was 14 per cent greater for the Light Silage group. With silage at $5.00, hay at $25.00 per ton, and grain the same ($52.35) the feed cost per 100 pounds of milk for the Heavy Silage group becomes $1.82 and that for the Light Silage group $1.80. A shift in the price of grain does not materially alter the spread in the cost between the total rations.

It is realized that these results will be received with much surprise and doubt by many advocates of heavy silage feeding. They should serve to stimulate the study of the adaptation of silage production to the conditions existing on a given farm and area. In so far as certain commercial interests are concerned, which in recent months have brought unscientific charges against silage in comparison with certain feed treatment processes, it is hoped that they will receive no impetus from these results in furtherance of their pernicious propaganda.

This experiment is being repeated this year under the same

conditions, essentially, so this paper may be regarded as a progress report.

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THLASPI ARVENSE (FRENCH WEED) IN RELATION TO DAIRY PRODUCTS*

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An especially obnoxious defect in cream and butter on several occasions has been brought to the attention of the authors. Investigation of conditions at one creamery showed several cream producers were delivering a product with the particular taste and odor in question. Indications pointed to the feed used as a source of the trouble. The grain ration contained a large proportion or grain screenings. The experiences of Canadian butter manufacturers and references in the literature, especially those of Canadian origin, suggested the possibility that the objectionable conditon might result from the seeds of the *Thlaspi arvense*, known under a variety of local names, but most commonly in Western Canada and in Minnesota as French weed. The presence of seeds of this specie in the screening feed used was demonstrated by the staff of the Minnesota Seed Control Laboratory. As the result of these observations, the experiments reported were initiated.

The literature on the subject is quite extensive, and numerous observations are reported, especially in Canadian publications, which indicate rather clearly the objectionable features of this plant. The plant is known by a variety of names including Penny cress in Europe; Mithradate mustard in Gray's Manual of Botany; Wild garlic; and Fanweed. In Manitoba and the Dakotas, it is generally called French weed or Stinkweed. It is a member of the mustard family.

The plant in appearance resembles the common Shepard's purse (*Bursa pastoris*), from which it is distinguished by the pods, which as a rule are about three-fourths of an inch across, flat, and circular, having a well defined wing around the margin and a

notch at the apex. The plant is both an annual and a winter annual. Seed formation begins by May and continues on plants of different ages until freezing weather in the fall. When moistened, the seeds have a nauseous garlic-like taste and a pungent odor. The green plant shows these same characteristics but to a less degree.

Authorities agree that the weed is an immigrant from Europe, where it is said to be especially common in France. The presence of *Thlaspi arvense* seeds in rape seed cake from India, mentioned by Werenskiold (1), suggests that this pest may also be found in India. Apparently, it made its way to the North American continent some years ago. Darlington (2) states that it was known in Michigan from 1860 to 1881.

In 1892, Waldron (3) listed it as a troublesome weed that had recently appeared in North Dakota. The same year, McKay (4) reported French weed as one of the especially troublesome weeds that had recently attracted attention in various parts of Canada. He also reported special trouble from it in Manitoba. Reports on the same subject were published by Clark and Fletcher (5). Howitt (6) in 1908 mentions it as one of the worst pests of the grain fields and states that one means of dissemination is through the agency of ground screenings and as an impurity in farm seeds.

These and other writers including Shaw (7) emphasize particularly the injury to the small grain crops caused by this pest but in some cases also mentioned the plant as giving a bad taste to milk and to the beef of animals which feed upon it. Other authors mention that it is necessary in certain localities to keep cattle off pastures containing this plant for from two to three weeks previous to slaughter in order that the carcass may be free from the objectionable taste.

French weed taint is quite common in cream received at butter factories in Manitoba and Sasketchewan, according to information received by correspondence from the Dairy Commissioners and members of the staff of the Agricultural Colleges in these Provinces. In these regions, the trouble appears most commonly early in the spring and sometimes late in the fall, presumably

from cows' eating the weed in question when the pasture grass is not abundant. It also appears that sometimes trouble is caused from animals eating the chaff and waste where grain has been threshed. Cases of French weed tainted cream occasionally appears during the winter in these Provinces, probably from the cows' eating hay containing the dried plant.

The French weed has been known in the Northwestern part of Minnesota for probably thirty-five years, but only in recent years has it become a serious pest. In 1928, the State department of Agriculture issued posters and other information calling attention to the obnoxious character of the weed. It is found at present generally distributed in the Red River Valley and to a considerable extent to the east and south in Minnesota.

The fact that this weed grows chiefly with small grain results in the presence of the seeds in screenings. Immense quantities of screenings are available in Minnesota on account of the extensive milling industry. A considerable part of the screenings secured from grain grown in the prairie section of Canada also find their way into the hands of the commercial organizations interested in the preparation of mixed feed in the United States. According to the Dominion Department of Agriculture, screenings to the amount of about 100,000 tons taken from Canadian export grain were shipped from the Lake ports into the United States in 1927.

As a result of the facts brought to light concerning the danger to dairy products from French weed seed, some commercial feed mixers have recently installed elaborate machinery to remove the French weed and common mustard seed from the screenings. This reduces the danger from the use of commercial feeds containing screenings. Undoubtedly in earlier years especially, the lack of care in grinding screenings was a factor in the spread of French weed as well as of other obnoxious species.

EXPERIMENTAL

The reports in the literature regarding the injurious effects of French weed upon dairy products are based upon general observations rather than controlled experiments. In order to

obtain accurate information, a series of experiments were conducted for the purpose of studying: (a) the effect of various amounts of French weed seed upon the quality of milk and butter; (b) the relation of the length of interval from the time consumed to the appearance of the objectionable taste and odor; (c) the effect of feeding the green French weed plant; (d) the efficiency of common practices for removing the objectionable flavor and odor; (e) the possibility of detecting by easily applied methods the presence of French weed seed in mixed feeds.

The French weed seed used was obtained from a commercial feed and seed company which has in use machinery designed to remove the seed of this weed from grain screenings. The material as obtained was found by the seed analyst of the State Department of Agriculture to contain 22 per cent of French weed seed. By the use of various screens and a fanning mill, it was possible to concentrate the French weed portion until it was reported to represent 50 per cent of the total. A closer concentration seemed impractical without hand picking. The remainder was composed of the seeds of pigweed, foxtail, lambs quarter, barnyard grass, and Canada thistle. These seeds did not have a pronounced taste and were therefore ignored. In giving the weights of French weed used in the experiments, it should be understood that an allowance is made for the foreign seeds present. The seed mixture containing the French weed was thoroughly ground to remove the danger of seed distribution through the alimentary canal of the animals and to imitate the conditions in which the seed usually is found in commercial feeds.

The ground seed was fed mixed with the regular grain mixture used for the University herd. It will not be necessary to report the numerous experiments in detail. It was found that the animals ate the feed mixture containing the ground seed freely the first time, but when fed a second lot, the same animal often ate the mixture with evident lack of relish. In some cases, the feed was refused entirely. This result was experienced when no more than 150 grams of the French weed seed was added to 8 pounds of mixed feed. In a later series, the French weed seed was mixed with feeding molasses and then with the grain. By this proce-

dure, the animal would readily consume the prescribed amount. It was noted that a strong odor of garlic appeared almost as soon as the feed was moistened in the cow's mouth. By the time the eating of the grain was completed, the typical odor of French weed could be detected as much as ten feet from the feed box. Shortly after the feed was consumed, the breath of the cow began to show a pronounced French weed odor. The pungent odor was sufficiently strong from the breath to be distinctly irritating to a person's eyes when near.

The milk was drawn either by hand or with a machine. It was examined carefully when fresh and again after standing in a closed container for at least 12 hours. The milk was then separated. After examination, the cream was churned and the butter examined from time to time for a period of about a month. It was found that for a cow producing milk at the rate of 30 pounds daily, 90 grams of French weed fed two hours before milking did not produce any noticeable effect. The intake was then increased to 200 grams, which was found to give a decided effect upon the milk, cream, and butter.

Experiments were next made to determine the effect of the interval between the ingestion of the French weed and occurrence in the milk. Intervals from 2 to 12 hours were used between feeding and milking. It was found that the maximum effect appeared about 3 hours after feeding; by 5 hours, the obnoxious condition had largely disappeared; by 12 hours, no effect whatever was noticeable. After determining that the 3-hour interval apparently gave the most pronounced results, later experiments were all made on this basis. Varying amounts were fed to the same and to different cows including some producing milk liberally and some with a small production. Some indications were obtained that a more pronounced result was obtained with cows producing a small amount of milk. With cows producing at the rate of 25 pounds daily, 150 grams of the seed fed 3 hours before milking gave a pronounced effect.

Our experiments indicate that the minimum amount that will ordinarily result in a taste and odor that will be observed is between 90 and 150 grams of the seed when fed at an interval favorable for the appearance of the objectionable condition.

Effects of feeding green French weed plant

Complaints received by the authors concerning butter defects apparently due to French weed have been confined to the winter season when dry feed is used. The source of the trouble must be the ingestion of the seed with the grain or the dried plant with the hay. Some evidence has been obtained that either may occur. Statements in the literature indicate that trouble has been most frequently experienced during the grazing period.

To test the relation of the green plant to the appearance of the obnoxious flavor and odor in butter, a plot was sown to the French weed and experiments made by feeding the green plants in varying quantities. Difficulty was experienced in getting the cows to consume the green plants even when the cows were receiving no other green forage and had, for this reason, a keen appetite for ordinary green feed. No results were obtained from 300 grams of the green plant fed 3 hours before milking. When 610 grams were consumed, the same interval before milking, the typical French weed taste was readily detected in the milk, and, as usual, more pronounced in the cream and the most pronounced in the butter. The same results were found with larger amounts. There seems to be no question but that the objectionable taste will appear if the cow consumes a sufficient amount of the green plant; however, the extreme dislike for the green plant shown by every one of the 8 cows used suggests that the only danger from the green plant is when a shortage of other green forage in the pasture compels the cow to devour the objectionable French weed. It is possible that certain animals may develop a taste for the plant and in time come to eat enough to affect the milk regardless of the presence of other feed.

The detection of French weed seed in mixed feed

To samples of the mixed feed in use at the University dairy barn, ground French weed seed was added in amounts ranging from 0.5 to 3.0 per cent. Water at a temperature of approximately 50°C. was added sufficient to moisten the entire mass. · In every case, the typical French weed odor was readily noticeable within a few minutes. The strength of the odor varied

with the amount of the ground seed added, but even with the 0.5 per cent sample, the odor could be readily noted and the irritating effects felt by the eyes. The odor from feeds containing 3 per cent of French weed was so pronounced that it was detected in the air of a large room by persons entering. The odor becomes most noticeable when the warm moist feed is enclosed for a short period in a partially filled container.

Commercial feeds composed partly of screenings often contain feeding molasses, largely to add to the palatability by covering up the bitter taste common to screenings. The odor of the molasses also obscures that of the French weed in making the test described. To remove this difficulty, the plan was adopted of washing the molasses from the feed and then noting if the typical odor of French weed could be observed. The procedure followed was to place about a pound of the feed to be tested in a cloth or towel of rather close texture. A gallon or more of warm water was poured, a portion at a time, on the feed and within a few minutes the free water was pressed out with the hands. The treatment was continued until the molasses appeared to be mostly removed. The washed feed was then put in a closed container and kept warm until examined within half an hour. By this means, the presence of French weed seed can be readily detected when even less than 0.5 per cent is present.

The simple plan of testing suggested will indicate the presence of French weed in quantities far below that apparently necessary to produce any serious taint in the milk. Our experiments indicate that a minimum of possibly 125 grams per day is necessary to produce noticeable flavor in the milk. If the screenings fed contained 2 per cent of French weed seed, 13 pounds would be required to carry 125 grams, which is suggested as the minimum likely to produce serious results in the quality of the milk.

Elimination of French weed taste

The prevention of food flavors in dairy products and the elimination of those that do gain access has attracted the attention of investigators. The work of Babcock (8) is of especial significance in this field. Apparently those flavors which are the result of

the presence of volatile oils are the most difficult to remove, presumably because they unite with the fat itself. A number of the common methods of reducing or eliminating feed flavors were tried with cream from the milk of cows, each receiving 225 grams daily of French weed in her grain ration. In each case, the treatment was applied to the cream before churning. The results reported are from an examination of the butter.

It was found that ordinary pasteurization of the cream somewhat reduced but did not eliminate the objectionable flavor. Affected cream was diluted with water and reseparated. The resulting butter was considerable better than the check, but the obnoxious flavor persisted. Neutralization of the cream after souring according to the procedure followed as a regular routine in many butter factories was without material effect. Heating the cream under vacuum, as practiced in a commercial way in some localities to remove objectionable odors and tastes, gave the best results but did not completely remove the French weed taste. The plan of using mineral oil, as recommended by McDonald and Crawford (9) was tried with the result that the French weed flavor was reduced but not eliminated.

A test was made to determine how small a percentage of French weed tainted cream might seriously affect butter quality. Tainted cream was obtained by feeding 227 grams of French weed daily to each of a group of cows. Cream from this milk was added to 3 lots of untainted cream at the rate of 6.5, 15, and 25 per cent. The 6.5 per cent of tained cream did not affect the butter so far as could be determined on examination by a Federal buttergrader, and it was scored 92. The lot containing 15 per cent of the tainted cream showed evidences of French weed flavor and was scored 91. The sample containing 25 per cent of the French weed cream showed more pronounced evidence of the objectionable taste and was scored 90.

Characteristics of the French weed taint

One of the interesting facts about French weed is the almost complete lack of any odor to the whole dry seed and the immediate development of an exceedingly disagreeable nauseating odor when

the ground seed is moistened. The unground seed develops the same odor when wet but not so quickly. Seeds of other plants of the *Cruciferae* show the same characteristic regarding the development of their typical volatile products in the presence of water.

One of the early investigators of this subject was Werenskiold (1). He states, as do later authorities, especially Gildermeister and Hoffman (10) that the seed of *Thalspi arvense*, as well as of other members of the mustard family, contain a glucoside known as sinigrin (myronate of potassium) and an enzyme known as myrosin. In the presence of water, the myrosin hydrolizes the glucoside known as sinigrin into mustard oil and other compounds. It appears that in the case of *Thalaspi arvense*, mustard oil and another compound, probably oil of garlic, are produced by the action of this enzyme. The mustard oil may be responsible for the irritating effect of the odor from French weed, but the oil of garlic gives the typical flavor.

An experiment by the authors indicated the correctness of this view. French weed seed and mustard seed were macerated and soaked over night. They were then distilled until 200 cc. of distillate was available for each. A series of cream samples were prepared and treated as indicated: (a) check; (b) 5 per cent of French weed distillate; (b), 5 per cent of mustard seed distillate; (d) 2 drops of mustard oil; (e) five drops of ether extract from ordinary garlic (*Allium sativum*). An examination of the butter churned from the five lots showed the odor as obtained from the French weed extract was not the same as that from mustard oil. Both showed the same irritating odor, but with the French weed this was combined with the same odor as that obtained from the common garlic.

DISCUSSION

The experiments reported show that either French weed seed or the growting plant may be responsible for an obnoxious taste and odor in the milk of a cow consuming these products. It might be expected that the trouble would be widespread in view of the extensive infestation of this weed in some districts in

Minnesota, the Dakotas, and the Prairie Provinces of Canada. The extreme distaste of livestock for the seed and forage is undoubtedly one explanation for the fact that more trouble does not occur. Apparently it is consumed only when the animal can not avoid doing so on account of the seed being in the grain mixture fed, or when the animal has a craving for green feed, and forage other than French weed is not available. This occurs usually in early spring and late fall. The common presence of this weed seed in grain screenings calls for care in feeding such products. The tests described offer a simple means of determining whether the objectionable seeds are present. Furthermore, the effect is observed only when what would appear to be a considerable amount, namely about 125 grams of the seed, is consumed. The effect of a smaller amount may be noted on the breath of the cow.

The experiments show that the French weed taint disappears completely within 12 hours after feeding. This indicates that the same practice as found effective in preventing the appearance of other food flavors will be effective, namely, feeding the suspected feed immediately after milking, with a reasonable certainty that the effects will be gone by the time for the next milking.

SUMMARY

Thlaspi arvense, known as penny-cress, French weed, or stinkweed, is a troublesome weed of the mustard family. It is the cause of a very objectionable taint in milk, cream, and butter.

This weed is a serious pest in the grain growing areas of Manitoba, Sasketchewan, Minnesota, the Dakotas, and Montana, and reports indicate that it is spreading.

The characteristic odor develops when the seed is in contact with water as the result of the action of an enzyme known as mycrosin, which hydrolyzes particular glucosides found in the plant. Apparently both oil of mustard and oil of garlic are formed.

It was shown experimentally that the ingestion by a lactating cow of French weed seed to an amount between 90 and 150 grams or of the green forage to the amount of 500 grams or more re-

sults in the characteristic taint in the milk. The odor is more marked in the cream than in the milk and is most pronounced in the butter. This may be taken to indicate that the volatile products unite chiefly with fat. The most pronounced results were obtained when the interval between feeding the seed and milking was 3 hours. The effect was reduced after an interval of 5 hours and completely gone by 12 hours. Feed containing French weed seed can be fed immediately after one milking without danger to the quality of the product from the next milking.

The common methods used to eliminate feed tastes from milk are only partially successful in removing the tainting of French weed. The taint could be detected in the butter from mixed cream when 15 per cent of the cream was tainted. French weed may be readily detected in mixed feeds in proportions as low as 0.5 per cent.

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GRADES AND METHODS OF ANALYSIS OF DRY SKIM MILK*

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The increased use of dry skim milk in the food industries has created a need for its proper classification by grades, so that the grade name may be taken as an index of quality. Formerly the quality of this product has been appraised by direct observation of its physical properties, by the results of selected laboratory determinations, or by a combination of both. Grading practices of the past have not been uniform due to the following: variation in the criteria accepted as a basis for grading; lack of adequate terminology to be applied to characteristics of quality; variations in laboratory practice; and undefined expression of the limits of probable errors in analysis.

The work of the Standards Committee of the American Dry Milk Institute consisted of collaborative work on laboratory technic, selection of the most suitable methods of analysis, the examination of a large number of samples and the correlation of the results of inspection and laboratory determinations with the more universally accepted trade demands of quality.

Three grades of quality have been established and these in turn are applied to each of three divisions named according to the method of their manufacture—spray, vacuum drum, and roller. A single standard for evaluating the quality of all dry skim milk would be simpler but owing to a variation in the relation of solubility to the several industrial applications of this product, the above division is deemed necessary at this time. Each of these divisions is subdivided into extra, standard, and third grade. It is not intended that classification under the

terms of spray, vacuum drum, and roller should be in any way prejudicial to a particular process.

NORMAL VARIATION IN COMPOSITION OF DRY SKIM MILK

	per cent
Butterfat	0.7 - 2
Moisture	2 - 5
Acidity (lactic, reconstituted basis)	0.09- 0.20
Total protein (N × 6.38)	30 -38
Lactose (by polariscope)	47 -53
Total ash	7 - 8

The variations in total protein, lactose and total ash indicated above appear to result from seasonal changes in the raw milk and are not generally considered as important factors affecting quality. Inasmuch as heavy metals, chemical compounds or substances deleterious to health do not usually contaminate the product during the process of manufacture, no consideration was given to their detection.

Requirements for Extra Grade

1. General requirements. All dry skim milk for human consumption shall conform in all respects to the Federal Food and Drugs Act of June 30, 1906, and all subsequent rulings and decisions relative to the same as issued, from time to time, by the United States Department of Agriculture.

The factory and factory equipment used in the manufacture of this product shall be maintained in a clean, sanitary condition. No person affected with any recognized infectious, contagious or communicable disease, or who resides, boards, or lodges in a household in which there is a person affected with such disease, shall be employed or permitted to work in or about any part of the factory in which dry skim milk is manufactured.

Dry skim milk shall be made from freshly skimmed milk to which no alkali or other chemical has been added and which has been pasteurized in the liquid state either before or during the process of drying, at a temperature of 145°F. for 30 minutes or its equivalent in bacterial destruction.

The powder shall be reasonably uniform in composition and the color shall be white or light cream and free from a brown or yellow color, typical of overheated stock, and free from any other unnatural color.

Upon reconstitution it shall have a good flavor and be substantially free from sediment and specks.

2. Detailed requirements.—

	EXTRA GRADE SPRAY DRIED— MAXIMUM	EXTRA GRADE VACUUM DRUM DRIED—MAXIMUM	EXTRA GRADE ROLLER DRIED— MAXIMUM
Fat........................	1 5 per cent	1 5 per cent	1 5 per cent
Moisture....................	4 0 per cent	4 0 per cent	4 0 per cent
Titratable acidity (reconstituted basis)......................	0.16 per cent	0 16 per cent	0 16 per cent
Solubility index..............	1 cc.	2 cc.	12 cc.
Bacterial count (reconstituted basis)......................	30,000 per cc.	30,000 per cc.	30,000 per cc.
Sediment.....................	No. 3	No. 3	No. 3

Flavor: In powdered form and on re-solution the products shall be sweet and clean and free from rancid, tallowy, fishy, cheesy, soapy or other decidedly objectionable flavors or odors.

Physical: It shall be free from all hard lumps and show no more black or foreign specks than in sediment no. 3.

Packing: The package shall be of such a character as to prevent contamination by dust, dirt or other foreign matter and to reduce to a minimum the possibility of absorption of foreign odors and moisture.

Requirements for Standard Grade

Any dry skim milk failing in one or more particulars to meet the requirements for Extra Grade shall be classed as Standard Grade, provided it meets all of the requirements for Standard Grade outlined below.

1. General requirements. Same as for Extra Grade.

2. Detailed requirements as follows:

	STANDARD GRADE SPRAY DRIED—MAXIMUM	STANDARD GRADE VACUUM DRUM DRIED—MAXIMUM	STANDARD GRADE ROLLER DRIED—MAXIMUM
Fat..........................	2.0 per cent	2.0 per cent	2 0 per cent
Moisture....................	5.0 per cent	5.0 per cent	5.0 per cent
Titratable acidity (reconstituted basis)....................	0.2 per cent	0.2 per cent	0.2 per cent
Solubility index..............	1.5 cc.	5 cc.	15 cc.
Bacterial count (reconstituted basis)....................	100,000 per cc.	100,000 per cc.	100,000 per cc.
Sediment...................	No. 4	No. 4	No. 4

Flavor: In powdered form and on re-solution it shall comply with the requirements for Extra Grade with the exception that a storage flavor is permissible.

Physical condition: It shall be reasonably free from hard lumps and any large number of black specks or foreign matter.

Requirements for Third Grade

Any dry skim milk failing to meet the several requirements for Standard Grade especially with reference to moisture, acidity, sediment, or bacterial count, or those in which the odor or flavor indicate partial decomposition shall be classed as Third Grade and considered unfit for human consumption.

Revision of Grades

The above limits for grades are exceptionally broad and it is anticipated that changes will be made from time to time when available data indicate such changes will improve the quality of the product.

DISCUSSION OF DETERMINATIONS RECOMMENDED AS A BASIS FOR THE APPRAISAL OF QUALITY

Butterfat. Dry skim milk is manufactured to contain a minimum of butterfat. Excessive amounts have been observed to cause the development of an undesirable tallowy flavor and odor during storage. The point of maximum tolerance indicated in

the grading schedule was obtained by noting the effect of varying butterfat content on the resultant flavor.

Moisture. It has long been observed that skim milk powder which is high in moisture at the time of manufacture gradually becomes more insoluble upon storage. Such powders develop stale, tallowy, rancid and other off flavors. They develop lumpiness and more readily decompose during storage and frequently become questionably fit for human consumption. As dry skim milk is very hygroscopic, it is important to store it in a dry atmosphere to maintain low moisture content.

Various methods for determining moisture have been used and a comparison of results seem to indicate that more concordant results can be obtained by using the Toluol distillation method indicated herein.

Acidity. The determination of total acidity has been used freely in judging the quality of milk and milk products. It has been assumed to be a reliable criterion of the degree of bacterial development and although the titration values are not an actual measure of free acidity, the increment above the recognized normal value for the milk or milk products usually represents free lactic acid. Although high acidity of dry skim milk is usually accompanied by other unfavorable characteristics, it is thought desirable to suggest a uniform procedure for the estimation of titratable acidity and to include a maximum in the grading scheme. When the acidity of the raw skim milk is close to the souring point the resulting dried product is insoluble and tends to undergo other degenerative changes during storage. The acidity of the powder is expressed in terms of reconstituted skim milk because of the ready analogy to figures obtained on the raw product. As the degree of dilution and the amount of indicator readily affect the determination, strict adherence to the method is seemingly essential if comparable results are desired.

Solubility index. This procedure involves, in a measure, speed of solution as well as solubility. Solubility is a characteristic which has been beset with uncertain interpretation and by some it is thought that a skim milk powder of high solubility more nearly approaches on re-solution the physical characteristics of

the original skim milk. On the other hand there are others who believe that an insoluble powder will, when given sufficient time, finally reach as close to the physical condition of raw skim milk as does the more soluble powder in the shorter period. Some of the consumers of dry skim milk require powders that are readily soluble in water while others do not recognize this as being an important factor in the satisfactory use of the product. Solubility is a recognized characteristic of dry skim milk powders and can only be considered as an indication of quality in terms of each type of powder.

The most accurate and technically correct method for establishing solubility is that proposed by Lampitt and Hughes. This method, however, lacks simplicity although it affords an ultimate standard which will serve as a basis for more accurate investigations. It is believed that a quicker method and one possessing reasonable accuracy would be more useful.

Bacterial count. It is not definitely known if moderate variations in bacterial count affect the quality of dry skim milk. In the interest of sanitary practice and public health, it is believed that the consideration of bacterial count should be included for grading purposes. It is admitted that excessively high counts indicate that the original raw product was of poor quality, or a delay in the manufacturing process favored undue growth, or that contamination took place during manufacture or packaging. All of these are undesirable conditions and it is believed that a product of relatively low count will be more resistant to decomposition and aging effect than will the product having a relatively high count. It is probable that such decomposition starts before the milk is dried and action is continued in the dry state as a result of enzymatic or catalytic action.

Sediment. The undesirability of visible discoloration of the product by burnt particles or dust is self-apparent. Some of the more critical uses of dry skim milk reveal the presence of sediments which cannot be detected in the dried product by mere physical inspection. Foreign particles may enter the process with the raw milk or be picked up during the manufacture while burnt particles usually result from too high temperatures being

used during the drying process or by the local adherence of milk deposits to certain areas in the drying equipment. The sediment testing procedure proposed is similar to that rather widely used for whole milk. In testing dry skim milk of low solubility, sediment particles are apt to be obscured by the insoluble protein. In view of this, an alternative procedure for such milks has been suggested employing the principle of quiet sedimentation.

Color. Variations in the color of dry skim milk powder are not wide. If the milk is scorched or otherwise discolored, the condition is noticeable in the powder and may appear in the reconstituted milk.

Flavor and odor. The appraisal of flavor and odor as might be expected is subject to elements of variation and inaccuracy. Competence comes to the individual with experience and the cultivation of practiced discrimination. It is felt that by providing descriptive terms for the several objectionable flavors found in imperfect dry skim milk the appraisal of flavor and odor may be carried out in a more exact manner and imperfections related to their probable cause.

METHODS OF ANALYSIS

1. Method of sampling

On account of the readiness with which dry skim milk absorbs moisture, it is very important that all utensils and containers used for sampling be *clean* and *dry* and that the work be done as speedily as possible. Samples should always be taken at points beneath the exposed surfaces. For complete laboratory analysis 1 pound samples are required.

When it is necessary to prepare a composite sample, in order that the final sample will be representative of a given quantity of powder, several fairly good sized portions should be taken at different points and immediately transferred to a dry container in which they can be thoroughly mixed.

In barrel sampling, it is suggested that a long tube made of brass or nickel (iron should not be used) one and one half inches in diameter and sharpened at one end be thrust the full length of

the barrel at a point on the surface one third the distance between the centre and the edge.

The container should be large enough to hold all of the material sampled and readily permit of mixing the sample as described under "Preparing the Sample for Analysis." After mixing, immediately transfer the final sample to the shipping container and fasten the cover tightly. Only single or double friction top cans are recommended for shipping samples. Variations which may occur in a given quantity of powder can be more readily detected by examining separate individual samples rather than a composite sample and, in some cases, this practice is preferable.

Important—

Do not sample unless hands and clothing are clean and dry.

Do not sample in a humid atmosphere or in a room into which steam is being discharged.

Do not sample in a damp cold storage room or with utensils or containers which have just been brought from a colder room into a warm room.

Do not sample when your attention is being distracted by other duties.

2. Preparing the sample for analysis

If the sample of powder completely fills the container in which it is shipped, transfer all of it to another clean, dry container of about twice the size. With a suitable sized spoon turn over the contents by lifting it from the bottom of the can, at the same time slowly rotating the can at an angle of 45°. Mix the powder in this manner, by turning over a spoonful every second during a period of one minute. It is important, during mixing, to take the powder out of the lowest edge of the can. Precautions should be taken to reduce, to a minimum, the absorption of moisture during mixing; and as soon as the mixing is completed the sample should be returned at once to its original container and covered tightly. The above procedure can also be used for preparing composite samples by the use of a larger spoon and a larger container.

3. Moisture—Toluol distillation method

Apparatus required. 1 moisture tester complete, Bidwell and Sterling type (see Eimer & Amend Catalogue No. 28600) which
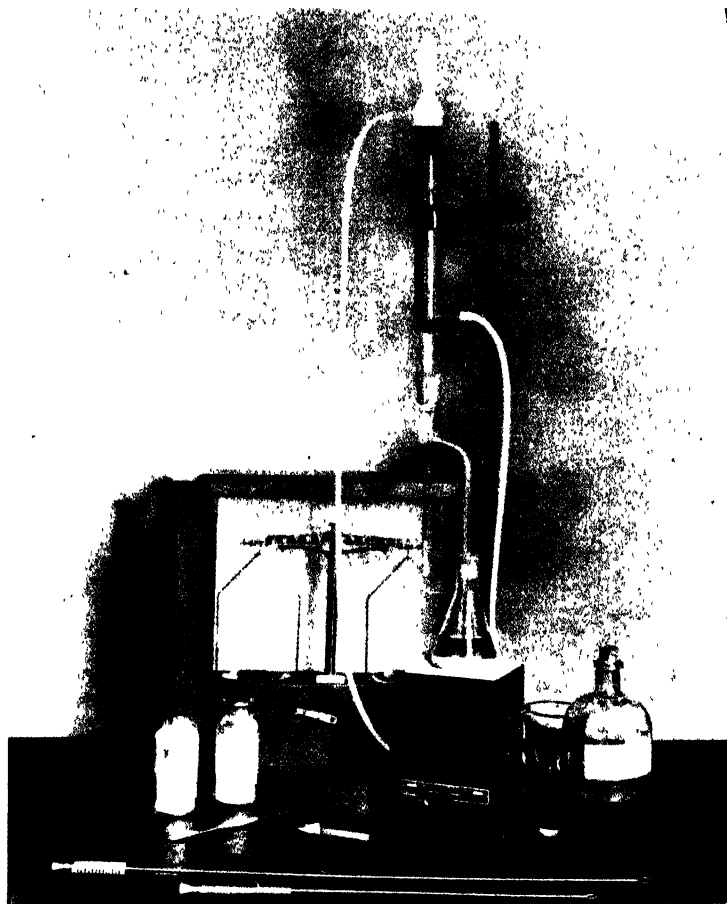
FIG. 1. EQUIPMENT FOR MOISTURE DETERMINATION

consists of a 250 cc. Erlenmeyer flask, 2—1 hole cork stoppers, distillation tube and condenser.

One electric hot plate or preferably electric heater (see Eimer & Amend Catalogue No. 24920).

Rubber tubing—suitable quantity for connecting water to the condenser (see Eimer & Amend Catalogue No. 30682—$\frac{1}{4}$ inside diameter). Ring stands and clamps for holding the apparatus.

Procedure. Introduce into the 250 cc. clean, dry Erlenmeyer flask 50 grams of spray process milk powder or 25 grams of roller process milk powder; immediately add sufficient toluol to cover the sample completely. Usually about 75 to 100 cc. is sufficient. Connect the flask with a condenser by means of the distillation tube. (For further information, see Ind. Eng. Chem., published February 25, 1925, page 147.)

Fill the receiving tube with toluol by carefully pouring through the top of the condenser. Bring to a boil rapidly by heating on the electric hot plate and then reduce the heat so that the Toluol will condense at the rate of about four drops per second. Thirty minutes after distillation has begun, dislodge any water particles in the condenser tube by means of a brush and wash down with 10 cc. of toluol. Twenty minutes later repeat and continue the distillation for an additional ten minutes. The flask should be shaken frequently after it is first put on the heater to prevent the powder from being burned and until the boiling is established and heat reduced to permit required rate of distillation.

Allow the receiving tube to come to room temperature. If any drops of water adhere to the side of this tube they can be forced down by means of a copper wire. Read the volume of water, estimating to hundredths of a cubic centimeter and calculate the percentage.

The condenser and receiving tube must be chemically clean in order to prevent any undue quantity of water adhering to the condenser and receiving tube. Wash with a solution of washing soda or soap powder and rinse with clear water. If necessary, fill with cleaning solution (sulphuric acid and potassium dichromate) and allow to stand over night. Rinse successively with water, alcohol, and ether and allow to dry in the oven. The tubes must receive this treatment after each determination, but the condenser usually once a week. If necessary, blank determinations should be made. It is important to use only moisture free Toluol and to make sure that the receiving tubes are accurately calibrated.

Note: The above method is to be regarded as the recommended one, but other methods which give comparable results may be employed. It has been determined that a vacuum oven operated at 100°C. in such a way that a current of air, dried by bubbling through sulphuric acid and admitted diagonally opposite the evacuation outlet at such a rate that the air is renewed every thirty seconds when the vacuum of the oven is maintained at a pressure of approximately 70 mm. of mercury, will give good checks with five hours of drying. Aluminum dishes 2 to 2½ inches in diameter, shallow form, with tightly fitting covers and one or two gram samples should be used. Equally good results have also been obtained with the vacuum oven at 100°C. operated under a pressure of 1 mm. of mercury for a period of four hours.

4. Butterfat

Apparatus required. Same as for Modified Roese Gottlieb or Mojonnier method.

Procedure. Weigh out 1 gram of the well mixed milk powder directly into a dry Mojonnier extraction flask, or in a suitable container whereby it can be easily transferred to a dry Mojonnier extraction flask or a dry Rohrig tube. If the latter is used, it should be of the Biesterfeldt type and should have a capacity of 20 cc. from the bottom up to the spigot. Add 8.5 cc. of warm water. Cork and shake vigorously until dissolved, warming slightly if necessary to room temperature. Add 1.5 cc. of strong ammonium hydroxide, shake again thoroughly. Add 10 cc. of ethyl alcohol 95 per cent, insert cork and shake again thoroughly. Add 25 cc. of ethyl ether, insert the cork and again shake thoroughly. Add 25 cc. of petroleum ether and shake as before. Allow the ether layer to separate by leaving the flasks or tubes at rest for twenty minutes or by centrifuging, turning the handle of the machine 60 times at a slow rate of speed. Pour or draw off the ether layer into a previously weighed aluminum dish. Evaporate on the hot plate or on a hot water bath at a temperature sufficient to allow complete evaporation, but not so high that spattering or too vigorous boiling will result. Add 4 to

5 cc. of alcohol to the residue left in the extraction flask or tube and mix thoroughly without inserting stopper. Add 15 cc. of ethyl ether, shake thoroughly, then 15 cc. of petroleum ether and again shake thoroughly. Separate the ether layer as before and pour off or draw off into the aluminum dish. Make a third extraction in exactly the same manner as the second, omitting any addition of alcohol. If necessary, carefully pour a few cubic centimeters of distilled water down the side of the tube just prior to pouring off third extraction to raise level of the aqueous layer, so ethers may be completely poured off. It is important that at no time should any of the aqueous layer be allowed to run into the aluminum dish.

The time required for shaking, after adding the first portion of alcohol and subsequent additions of either ethyl or petroleum ether should be not less than one-half minute, providing the shaking is very vigorous.

Each time after pouring off the ether layer, the lip of the extraction flask or spigot should be rinsed with petroleum ether, allowing the rinsings to run into the aluminum dish. Petroleum ether should have a boiling point below 60 degrees and both petroleum and ethyl ether should be free from residue on evaporation.

After the ether is entirely evaporated from the aluminum dish, it should be placed in the Mojonnier oven for five minutes with the temperature at exactly 135°C. or in boiling water oven for thirty minutes or longer, as required, to bring it to constant weight. In weighing the dishes both when empty and when containing the extracted fat, they should be at a temperature preferably within 1° of the temperature of the balance; and should never have more than 2° difference. Fresh. sulphuric acid appears preferable as drying medium in the desiccator in which the dishes are cooled. If desired, confirm the purity of the fat by dissolving in a little petroleum ether. If a residue remains, remove fat completely by solution and washing out dish with petroleum ether. Dry the residue and deduct weight. Blank all reagents.

5. *Solubility index*

Apparatus required. A supply of 50 cc. conical sediment tubes specially graduated in one-tenth divisions from 0 to 1 cc., in 0.2 divisions from 1 to 3 cc., and 0.5 divisions from 3 to 15 cc., and also at the 50 cc. mark.

Suitable glass syphon tubes.

Hamilton Beach electric mixer.

FIG. 2. EQUIPMENT FOR SOLUBILITY INDEX

Two hundred or 300 cc. glass beakers or glass tumblers of equal capacity.

Fifty cubic centimeter wide mouth pipette.

Wood adapter or rubber pad to hold the conical sediment tube in centrifuge cup.

Procedure. Add 20 grams of dry skim milk to 200 cc. of distilled or filtered tap water at a temperature of 60°F. and imme-

diately agitate vigorously by the use of a Hamilton Beach electric mixer for thirty seconds. If necessary transfer immediately to a suitable sized container so that after the foam has separated it can be readily removed from the surface by means of a spoon. Allow to stand at room temperature for a period of five minutes and then remove all the foam from the surface with a tablespoon. Agitate the milk thoroughly with the spoon by stirring rapidly for fifteen seconds and immediately pour 50 cc. into the specially calibrated conical tube. Place the conical tube at once in a hand or motor driven centrifuge and whirl for fifteen minutes at the normal R.P.M. Syphon off the supernatant liquid to 7 cc. mark if possible. If the amount of sediment is so large that this cannot be done, then syphon it within 2 cc. of the surface of the sediment, being careful not to draw any of the sediment into the syphon tube. Add about 25 cc. of distilled or filtered tap water, temperature 60°F., and shake thoroughly so as to dislodge all of the sediment from the lower portion of the tube. If necessary use a metal wire to dislodge the sediment. Fill to the 50 cc. mark with distilled or filtered tap water, temperature 60°F., invert and shake the tube so as to mix the contents thoroughly and whirl a second time in the centrifuge for a period of fifteen minutes. Hold the tube in an absolutely vertical position so that the upper level of the sediment is in line with the eye. Read the amount of sediment in the tube and record the results in cubic centimeters. The upper surface of the sediment is easily seen when the tube is held toward a strong source of light.

Note: It is important that the water used in determining solubility should be free from sediment and always at a temperature of 60°F. Periods of stirring and centrifuging must also be observed exactly if reasonable checking results are desired. Do not use a steam turbine centrifuge and frequently check the speed to make sure that normal R.P.M. is maintained. The normal R.P.M. of the Babcock centrifuge varies according to the diameter of the wheel according to the following table:

Diameter of wheel	Normal R.P.M.
10 inches	1074
12 inches	980
14 inches	909
16 inches	848
18 inches	800
20 inches	759
22 inches	724
24 inches	693

6. Titratable acidity (reconstituted basis)

To 200 cc. of neutral or distilled water, add 20 grams of dry skim milk. After the powder is apparently in solution, withdraw by means of a pipette 17.6 cc. and transfer to a white porcelain cup, or similar container. Rinse out the same pipette with 17.6 cc. of neutral or distilled water and add it to the skim milk in the cup. Then add 0.5 cc. of a 0.5 per cent solution of phenolphthalein and titrate with N/10 sodium hydrate until a faint pink color persists for thirty seconds. The number of cc. of sodium hydrate used divided by 20 indicates the percentage of acidity of the reconstituted skim milk in terms of lactic acid. The phenolphthalein solution should be prepared by dissolving one-half gram of dry phenolphthalein powder in 50 cc. of ethyl alcohol and adding 50 cc. of water after the powder has gone into solution.

7. Foreign sediment and black specks[1]

Apparatus required. Wisconsin sediment tester using $1\frac{1}{4}$ inch disc.

Supply of $1\frac{1}{4}$ inch cotton discs.

Twelve ounce Bell top tumblers (2 inches diameter at base).

Procedure. One pint of restored milk containing 10 grams of the powder in 100 cc. should be passed through a $1\frac{1}{4}$ inch disc sediment tester and the disc examined, for foreign sediment, burnt particles, and compared with standard disc illustrations. If re-dissolved powders will not pass through the sediment tester, it

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[1] On account of difficulty in printing disc charts, photographic copies will be supplied on request to the American Dry Milk Institute, Inc., 221 North La Salle Street, Chicago, accompanied by 80 cents to cover cost.

is suggested that a one-half pint sample be allowed to stand in a 12 ounce bell-top tumbler (diameter of base 2 inches approximately) for five hours and amount of sediment in the bottom of the tumbler be compared with the standard tumbler illustration.

8. Flavor and odor

The dry skim milk should be examined for flavor and odor one hour or more after restoring to normal liquid form. It should be classified as: good, fair, or bad, off odors and flavors being characterized by such terms as—storage, stale, tallowy, rancid, cheesy, putrid, cowy, fishy, metallic, cooked or flat.

9. Bacterial count (reconstituted basis)

A. Completely dissolve 10 grams of a well mixed powder in 100 cc. of sterile, distilled water. It is suggested that this be done by weighing 10 grams directly into a 6 ounce sterile bottle containing 100 cc. of sterile water and furnished with a sterile rubber stopper. It is important that the mixture be thoroughly shaken so as to make sure that the powder is completely dissolved.

B. After reconstitution as in A, follow the procedure as outlined for the microscopic colony count (plate method) as published in the Standard Methods of Milk Analysis of the American Public Health Association, Fifth Edition, 1927. It is important to note that the final count is to be expressed as the plate count per cubic centimeter of the reconstituted solution.

ACCURACY OF RESULTS

It is not infrequent that in noting the results of laboratory analyses one observes that variations occur when duplicate determinations are made. He is prone to feel that the analyses cannot be relied upon unless results check exactly. The fact that the method of analysis has been recommended by a Committee who have given the details of the method careful consideration and study does not insure the fact that it is absolutely exact. It simply indicates that it is the best method they are able to recommend. In order that one may appreciate the limitations

of results that can be obtained upon duplicate analysis, a study has been made of the possible variations that may be expected and for information and guidance they are indicated as follows:

a. When determinations are made on the same sample in a given laboratory:

1. Moisture should check within 0.2 per cent.

2. Fat should check within 0.1 per cent.

3. Acidity of reconstituted skim milk should check within 0.01 per cent.

4. Solubility Index should check within:

0.1 cc. where the total sediment is not greater than 1 cc.

0.1 cc. to 1 cc. where the total sediment is between 1 cc. and 10 cc. The greater the sediment the greater the variation.

2 cc. where the total sediment is above 10 cc.

b. When determinations are made on duplicate samples in different laboratories:

1. Moisture should check within 0.4 per cent.

2. Fat should check within 0.3 per cent.

3. Acidity of reconstituted skim milk should check within 0.02 per cent.

4. Solubility Index should check as above.

The method of determining bacterial count has been adopted because it is the procedure generally followed for making bacterial counts in all dairy products. Wide variations in count are frequently noted and it is suggested that two or more determinations be made each on a different sample.

STUDIES ON THE CHEMICAL COMPOSITION OF BOVINE BLOOD*

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With the development of methods for the analysis of blood, a valuable tool has been placed in the hands of experimenters in physiology and nutrition and of physicians in the clinical laboratory. At the present time a great deal of information is available on the abnormal composition of human blood in disease. A knowledge of the composition of normal blood was obviously a preliminary necessity for the interpretations of these abnormalities.

In order to extend the use of blood analysis to cattle, normal values must first be established for the various blood constituents. A considerable amount of data along this line are available at the present time. Abderhalden (1) in 1898 published a rather complete analysis of the blood of various domestic animals including cattle. More recently important contributions have been made by Meigs, Blatherwick and Cary (22), Blatherwick (6), Robinson and Huffman (23) and Hayden and his co-workers (18, 19, 20). In the course of our study on the composition of the blood of dairy cattle under various conditions we have accumulated considerable data on normal animals which we have assembled in this paper. It is hoped that these data may add to the present knowledge of normal bovine blood and may be of value to future workers in the field of bovine physiology, nutrition, and pathology.

METHODS

Blood samples were obtained from the jugular vein with as little stasis as possible. Two samples were always taken, one in a

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small Erlenmeyer flask, with no oxalate, for the preparation of serum in which calcium and phosphorus were determined; the other in a large test tube under paraffin oil, using 40 mgm. of lithium oxalate to prevent clotting. About 75 cc. of blood were collected in this manner to be used for the determination of hemoglobin, carbon dioxide and the substances included in the Folin-Wu system of blood analysis. The portion of the sample used for the determination of carbon dioxide was centrifuged immediately under paraffin oil and the plasma removed.

Hemoglobin and carbon dioxide were determined by the gasometric methods of Van Slyke (25, 26). For the estimation of non-protein nitrogen, creatine, creatinine, uric acid, sugar and chlorides, a protein free filtrate was prepared according to the procedure of Folin and Wu (12). Non-protein nitrogen, creatine and creatinine were determined by the original procedure of Folin and Wu (12). Uric acid was determined by Benedict's method (5), sugar by the method of Folin and Wu (13) and chlorides by the method of Whitehorn (28). Urea nitrogen was determined on whole blood by the method of Van Slyke and Cullen (27). Phosphorus was determined in three forms, namely, inorganic, hydrolyzable organic and total acid soluble phosphorus, using the Briggs modification (7) of the Bell-Doisy method (2) with some slight modifications of procedure. Calcium was determined by Clark and Collip's modification (8) of the Kramer and Tisdall method (21, 24).

<center>EXPERIMENTAL</center>

The work embodied in this report includes the analysis of 59 samples of blood taken from the dairy herd of the Pennsylvania State College. The animals include calves and adults of various ages and breeds. The animals received the regular ration used for the dairy herd. The blood samples were taken during the late summer, fall, and winter and no attempt was made to study seasonal variations. The results, which are averages of duplicate determinations, are presented in tables 1 and 2. The animals are grouped into 4 groups on the basis of age. In the first group are those animals less than one month of age. In the second group

TABLE 1

Showing the composition of the blood of normal dairy cattle

ANIMAL NUMBER	SEX	BREED	AGE	WHOLE BLOOD								SERUM				PLASMA
				Hemoglobin	Non-protein nitrogen	Urea nitrogen	Uric acid	Creatine	Creatinine	Sugar	Chlorine as sodium chloride	Phosphorus Inorganic	Phosphorus Inorganic plus hydrolyzable organic	Phosphorus Total acid soluble	Calcium	Plasma bicarbonate CO₂
				per cent normal	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	volumes per cent
Animals ranging from 1 to 28 days of age																
1312	M.	A.	0–0–1		30 80	4 40	1 97	4 81	1 62	123 0	503					
1308	M.	H.	0–0–6		25 91	15 63	1 59	4 67	1 55	96 0	494				12 93	
1310	M.	H.	0–0–7		35 42		2.40	4 42	1 82	89 0	473				13 40	
1323	F.	A.	0–0–9	121 7	42 14	19 80	3 10	4 06	1 49	105 7	457	5 23	5 72	7 80	12 52	52 2
1327	F.	H.	0–0–10		26 99	8 21	1 53	3 83	1 73	99 8	440				13 46	
1321	F.	A.	0–0–11	104 0	37 15	10 53	2 19	3 35	1 47	99 8	471	5 05	7 60	8 44	11 76	58 9
1309	M.	A.	0–0–13		31 55	10 53	2 13	5 57	1 41	98 4	492	4 55	5 75	6 05	12 88	62 6
1307	M.	H.	0–0–16	90 8	30 77	10 00	1 76	4 07	1 63	105 0	505				12 60	
1324	F.	G.	0–0–17	99 2	20 67	12 68	1 71	3 65	1 54	138 0	426	5 30	7 90	8 57	15 00	65 5
1323	F.	A.	0–0–28	96 7	26 97	15 10	1 91	3 33	1 57	86 8	434	5 20	6 45	8 03	14 48	62 6
Animals ranging from 1 to 5 months of age																
1321	F.	A	0–1–7	79 8	27 62	15 59	1 64	4 78	1 61	104 5	479	6 17	7 50	8 47	14 49	65 5
1308	M.	H.	0–1–29		29 92	8 62	2 21	4 27	1 38	81 6	515	3 52			12 87	64 5
1000	M	H.	0–2–		26 64	14 05	2 86	2 49	1 53	64 3	536					61 7

1168	F.	H.	0- 2-	89.9	33.38	6.21	2.96	4 08	1.52	119 5	448	5 36	6.85	8.24	16.18	64.5
1169	F.	H.	0- 2-	105.4	34.47	12.99	2 00	4 95	1.60	142.8	460	4.77	6.57	7.59	13.81	56.0
1182	M.	H.	0- 2-	85.4	36.82	11.30	1 70	4 58	1.94	138 6	506				11.19	76.8
1168	F.	H.	0- 2-	98.5	31.86	7.12	1 94	6 99	1.45	90.5	487	5.72	7.70	7.91	12.64	65.3
1169	F.	H.	0- 2-	100.9	30.88	10.08	2.00	6.86	1 28	100.0	514	5.15	7.08	7.77	11.49	58.7
1170	F.	H.	0- 2-	95.1	31 58	7.57	1.85	5.83	1.60	120 7	461	3.95	6.28	7.07	11.59	73.0
1307	M.	H.	0- 2-		25.73	7.99	1.55	4.32	1.36	71.7	505	3 09			11.99	59.8
1296	M.	A.	0- 2-		30.52	12.79	2.00	4 25	1.27	77 0	501				14.30	
1292	F.	J.	0- 2-14	70.3	29.21		1.56	4 48	1 43	73 0	495				12.62	65.5
1302	M.	H.	0- 2-15		27.90	18.91	1.70	4.01	1.42	78 3	546	3.29	3.47		12.96	71.8
1303	M.	H.	0- 2-15		26.38	21.64	1 63	4.37	1 42	77.7	546	3.32	3.46		13.31	67.1
1301	F.	H.	0- 2-21	79.4	26 83	13.55	2.08	4 34	1.37	119.9	492	3.41			11.61	67.3
1293	F.	A.	0- 2-22	79.4	29.30	14.30	2 20	7.78	1 45	87.2	540				12.60	52.5
1291	M.	J.	0- 2-25	93.6	26.52	11.70	1.54	3 67	1 28	82 0	495					58.6
1292	F.	J.	0- 2-28		25.00		1 90	4 15	1.25	71.0	506				12.24	
1293	F.	A.	0- 2-28		23.60		1 92	3 67	1.41	80.0	510				10.00	
1290	F.	A.	0- 3- 6	73.3	22.20		1.80	3.89	1 32	72.2	499				11.62	55.1
1288	M.	A.	0- 3-14	86.9	28.00	11.20	1 95	3.61	1 35	75.0	490				10.55	56.7
1287	M.	A.	0- 3-18	86.9	30.18	12.41	1 85	3 83	1.36	67.8	487				12.20	59.8
1290	F.	J.	0- 3-23		25.75	11.00	2.05	2 90	1.19	93 0	513					
1291	M.	A.	0- 4- 5		25.00	10.43	1 96	3.90	1 33	87.3	470				13.80	
1287	M.	A.	0- 4- 8		24.96	11.10	2.05	4 54	1.31	69.0	488				14.16	
1283	F.	A.	0- 4- 8	77.1	27.96	11.99	1.65	3 99	1.32	71.8	496				11.00	63.6
1280	M.	J.	0- 4-15	81.4	26.91		2.20	4 25	1.45	75.0	491				9.96	64.3
1283	F.	A.	0- 4-22		27.48	13.40	1 68	4 47	1.25	66 0	495				11.70	
1280	M.	J.	0- 5- 5		27.53	10.00	1.45	4 04	1 21	82 2	488				11.76	

Animals ranging from 6 to 10 months of age

1292	F.	J.	0- 6-12	101 2	33 06	13.93	3.22	4 42	1.38	59 6	506	5 45	6.70	7 50	12.55	54.1
1271	F.	G.	0- 6-16	86.2	34.40	15.00	1.71	7.11	1.33	65 5	511	3 50			12.20	57.5
1280	M.	J.	0- 6-17		29.13	14.28	1.72	4 06	1.36	90 0	506				13.40	61.7

TABLE 1—*Concluded*

ANIMAL NUMBER	SEX*	BREED*	AGE*	Hemoglobin (per cent normal)	Non-protein nitrogen (mgm. per 100 cc.)	Urea nitrogen (mgm. per 100 cc.)	Uric acid (mgm. per 100 cc.)	Creatine (mgm. per 100 cc.)	Creatinine (mgm. per 100 cc.)	Sugar (mgm. per 100 cc.)	Chlorine as sodium chloride (mgm. per 100 cc.)	Phosphorus Inorganic (mgm. per 100 cc.)	Phosphorus Inorganic plus organic hydrolyzable (mgm. per 100 cc.)	Phosphorus Total acid soluble (mgm. per 100 cc.)	Calcium (mgm. per 100 cc.)	CO_2 Plasma bicarbonate (volumes per cent)
								Animals ranging from 6 to 10 months of age—Concluded								
1271	F.	G.	0- 6-27		31.90	19.07	2.13	3.56	1.26	78.0	482				11.76	
1266	F.	J.	0- 8- 7		26.43	14.65	1.80	3.89	1.24	67.0	458				11.65	
1265	F.	H.	0- 8-10		32.43	16.42	1.62	3.82	1.26	76.5	481				12.80	
1325	F.	H.	0- 8-10	110.2	31.45	10.08	2.32	4.54	1.51	127.0					14.70	38.1
1266	F.	J.	0- 8-14		35.72	20.00	2.10	2.98	1.30	75.0	491				12.46	
1259	F.	G.	0- 8-19	102.1	42.10	18.00	1.92	4.77	1.39	67.0	480				11.83	61.6
1254	F.	J.	0- 8-29		26.78	13.90	1.52	3.72	1.21	69.6	471				12.60	
1259	F.	G.	0- 9- 0		35.20	17.00	2.10		1.38		478				12.87	
1254	F.	G.	0- 9- 6		35.98	17.93	1.91	3.41	1.32	70.0	476				12.06	
1250	F.	H.	0- 9-18		32.93	14.50	1.50	3.77	1.30	73.8	528				11.50	
1250	F.	H.	0-10- 2		25.60	13.97	1.90	4.08	1.45	74.0	510				11.60	
								Animals ranging from 1 to 9 years of age								
1237	F.	G.	1- 0- 4	81.1	29.20	13.30	2.28	4.05	1.41	68.4	515				12.87	56.0
1233	F.	J.	4- 2-27		34.20	13.30	2.40	3.83	1.37	51.0	509	3.75	5.10	5.75	13.40	38.1
1233	F.	J.	4- 3-25	109.0	32.20	13.52	2.49	5.01	1.66	47.0	532				14.78	55.1
1011	F.	B.S. + J.	4- 9-19	91.8	35.76	9.18	2.20	2.80	1.48	45.7		3.50	3.90	5.30	12.46	31.5
1011	F.	B.S. + J.	4-11-17	92.4	32.20		2.47	4.11	1.65	43.2					13.49	36.2
838	F.	J.	9- 3-10	103.6	29.23	10.26	2.92	3.79	1.31	51.8					12.88	

* M. = male; F. = female; A. = Ayrshire; H. = Holstein; G. = Guernsey; B.S. = Brown Swiss; J. = Jersey.
The final numbers refer to age in years, months and days.

are animals from one to five months of age. The third group includes those from six to ten months of age, and the last group includes animals from one to nine years of age.

In table 2 is given a summary of results given in table 1, with averages for various age groups, for male and female, and for the different breeds. The results obtained by other investigators are also given in this table. The results of other investigators have been recalculated in some cases so that all the data presented in table 2 might be comparable.

Hemoglobin. Tables 1 and 2 show the results of 29 analyses of blood for hemoglobin. The results are expressed as per cent normal and the values range from 70.3 to 121.7, the average value being 92.9. The hemoglobin content appears to be highest in the animals less than one month of age. Females show a higher value than males and Holsteins show the highest average of the various breeds. Our results agree well with those of Hayden and Fish (18).

Non-protein nitrogen. Our figures for non-protein nitrogen are in very close agreement with those of other investigators. Fifty-nine determinations give an average of 30.07 mgm. per 100 cc. of blood with values ranging from 20.67 to 42.14 mgm. The averages for the various groups of animals are all very close to the total average.

Urea nitrogen. Fifty estimations in the course of our work have given an average of 12.94 mgm. of urea nitrogen per 100 cc. of blood, with minimum and maximum values of 4.40 and 21.64 mgm. respectively. The low value of 4.40 mgm. was obtained on a sample from a calf one day old. The averages for the various groups differ only slightly from the grand average except in the case of the Brown Swiss-Jersey cross. It should be noted that this value of 9.18 is for one individual. Our results agree with those of most of the other investigators.

Uric acid. For 59 determinations of uric acid, using the direct method of Benedict (5), we report an average of 2.08 mgm. which exactly duplicates the findings of Hayden and Sholl (19). Our values range from 1.50 to 3.22 mgm. The only group of animals which differs decidedly from this value is that of the older animals,

TABLE 2

Showing a summary of the data given in table 1 and also the results obtained by other workers

	WHOLE BLOOD								SERUM				PLASMA
	Hemoglobin	Non-protein nitrogen	Urea nitrogen	Uric acid	Creatine	Creatinine	Sugar	Chlorine as sodium chloride	Phosphorus			Calcium	Plasma bicarbonate CO_2
Description									Inorganic	Inorganic plus hydrolyzable organic	Total acid soluble		
	per cent normal	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	Volumes per cent
Average for all animals	92.9	30.07	12.94	2 08	4.30	1 42	84.1	492	4.46	6.12	7.46	12.63	58.9
Maximum for all animals	121.7	42 14	21.64	3.22	7.78	1.94	142 8	546	6.17	7.90	8.57	16.18	76.8
Minimum for all animals	73.3	20.67	4.40	1.50	2.49	1.19	43.2	426	3.09	3.46	5.30	9.96	31.5
Average for animals less than 1 month of age	102.5	30.83	12.04	2.03	4.16	1.58	105.0	469	5.06	6.68	7.78	13.22	60.4
Average for animals from 1 to 5 months of age	86.9	28.28	11.91	1.91	4.49	1.40	87.6	499	4 54	6.11	7.84	12.40	63.3
Average for animals from 6 to 10 months of age	99.9	32.36	15.62	1.96	4.15	1.33	76.4	491	4.48	6.70	7.50	12.42	54.6
Average for animals from 1 to 9 years of age	95.6	32.13	11 56	2.46	3.93	1.48	51.2	519	3.62	4.50	5.52	13.31	43.4
Average for males	86.8	28.83	12.07	1.91	4.19	1.45	89 4	501	3.34	3.47		12.62	63.9
Average for females	93.7	30.71	14.19	2.05	4.34	1.41	81.9	487	4.92	6.51	7.46	12.65	56.7
Average for Ayrshires	89.1	29.14	11.69	2 01	4.30	1 40	87.2	491	5.47	6.26	7.59	12.48	59.0
Average for Holsteins	98.7	30.19	10.73	1.96	4.51	1.51	99.2	496	4.24	6.13	7.84	12.73	63.1
Average for Guernseys	95.8	32.78	16.61	1.96	4.63	1.38	72.6	481	5.30	6.57	8.57	12.66	61.5
Average for Jerseys	91.5	29.06	13.27	2 06	4.01	1.34	70.1	491	4.23	6.57	6.63	12.62	54.3
Average Brown Swiss-Jerseys	92.1	33.98	9.18	2.34	3.41	1.57	44.5	532	3.50	3.90	5.30	12.98	43.3
Hayden and Fish (18)	89.24	29.79	12.21	{2 04 / 0.623*}		1.20	46.52	444.1	5.7		9.29	10.88	

Folin and Dennis (10)............	24.00	14 00	0.2*						
Greenwald (14).................	32.00								
Hayden and Tubangui (20)......	31.00	18.8	2 12	1 84	96.00				
Hayden and Sholl (19).........	30.11	11.8	2.08	1.37	51.75	516.1			
Benedict (4)...................			0 5*						
Folin and Dennis (11).........			10.44	2.00					
Greenwald and McGuire (15)......			{ 7 23 / 2 35†	2.58 / 1.61†					
Abderhalden (1)...............						507.6	8.47	11.94	
Robinson and Huffman (23)......						542.8	5.87	11.0	59.2
Hart, et al. (16).............							3.94	16.25	
Meigs, Blatherwick, and Cary (22).....							5.38	10.38	
Hart, Steenbock, and Humphrey (17)..								10.00	
Blatherwick (6)...............									{ 61.5‡ / 73.0§

* Indirect method.
† Their own method.
‡ Cows.
§ Calves.

from one to nine years of age, where an average of 2.46 mgm. was found.

Our results check well with those of investigators who have used the direct method for the determination of this constituent. Folin and Dennis (10), using a method in which the uric acid is isolated from a blood filtrate as the silver salt before being determined colorimetrically, report a much lower value of 0.2 mgm. Benedict (4) using his modifications (3) of this technique obtained a value of 0.5 mgm. He pointed out that the uric acid determined by this method is "free" uric acid and that this is only a small fraction of the uric acid present. The remainder exists in a "combined" form from which it is easily set free by hydrolysis with acid. Davis and Benedict (9) have shown that the uric acid is combined with a pentose. After hydrolysis of a protein-free blood filtrate, he obtained values as high as 6.7 mgm. It should therefore be pointed out that our results and those of the other investigators whose results are reported in table 2 very likely do not represent true uric acid values but only that which is set free under the conditions of the analysis.

Creatine. In our work, we have found an average of 4.30 mgm. of creatine per 100 cc., with values ranging from 2.49 to 7.78 mgm. The oldest animal group showed the greatest variation from the average, with an average value of 3.93 mgm. The low value of 3.46 for the Brown Swiss-Jersey cross is possibly not significant since there is only one individual in this group. It should be noted that we have reported actual creatine values and not creatine plus creatinine.

Our results do not agree with those of other investigators. Folin and Dennis (11) report 10.44 mgm. and Greenwald and Mc-Guire (15), using Folin's technique, report 7.23 mgm. The latter investigators feel that Folin's method gives high results and they report a value of 2.35 mgm. using a new method.

Creatinine. From 59 determinations for creatinine we have obtained an average of 1.42 mgm. per 100 cc. with values ranging from 1.19 to 1.94 mgm. With the calves the values decrease with age. Holsteins give the highest average and Jerseys the lowest. There is considerable variation in values reported by other investigators.

Sugar. Our work has given an average of 84.1 mgm. of sugar for 58 determinations with values ranging from 43.2 to 142.0 mgm. A rather striking feature of the data is that blood sugar values decrease as the age of the animal increases. The youngest group gave an average of 105.0 mgm. while the group of older cows gave an average of 51.2 mgm. In other work we have always found low blood sugar values with cows which are lactating heavily. Our results for cows agree very closely with those found by Hayden and Scholl (19).

Chlorides. As a result of 55 analyses, we report an average of 492 mgm. of sodium chloride per 100 cc. The results range from 426 to 546 mgm. The animals in the group less than 1 month of age gave an average of 469 mgm. which is the group showing the greatest variation from the total average which we report. The value of 532 mgm. for Brown Swiss-Jersey is the result for one animal. Our average is about an average of results reported by other investigators.

Inorganic phosphorus. As a result of 20 analyses, we obtained an average of 4.46 mgm. of inorganic phosphorus per 100 cc. of serum, with values ranging from 3.09 to 6.17 mgm. The conspicuous thing about these results is that as the age increases the phosphorus values decrease. The group of youngest calves gives an average of 5.06 mgm., while the cows give a value of 3.62 mgm. per 100 cc. of serum. Females give a higher average than males and Ayrshires and Guernseys give higher averages than Holsteins and Jerseys. There is considerable variation in the values reported by other investigators.

Inorganic plus hydrolyzable organic phosphorus. In the work reported on blood phosphorus, we have found that in most cases only inorganic phosphorus has been considered. Meigs, Blatherwick and Cary (22) have reported on phosphorus distribution in blood plasma, but the distribution is not analogous to that which we report.

As a result of 16 determinations of inorganic plus hydrolyzable organic phosphorus in blood serum, we have found an average of 6.12 mgm. per 100 cc. with values ranging from 3.46 to 7.9 mgm. Here, again, the very young animals showed higher values than

the more mature ones. The average for females is nearly twice that for males. By deducting the average inorganic phosphorus from the average of the combined forms, we get 1.17 mgm. for the average hydrolyzable organic phosphorus in 100 cc. of blood serum.

Total acid soluble phosphorus. Hayden and Fish (18) report an average of 9.29 mgm. of acid soluble phosphorus per 100 cc. of serum as a result of 29 determinations on cows.

From 14 determinations we report an average of 7.46 mgm. of total acid soluble phosphorus per 100 cc. of serum, with values ranging from 5.30 to 8.57 mgm. Our results show a considerably higher average for calves than for older animals. Our results on cows are much lower than those reported by Hayden and Fish (18).

From all our results on the various forms of phosphorus in blood serum, it is clear that the serum of young growing animals has a higher phosphorus content than that of mature animals. This is to be expected when one considers the phosphorus requirement for bone-building in young animals. The lower phosphorus content of the serum of mature cows is also reasonable when one considers the large amounts of this element which are secreted into the milk.

Calcium. The results which we report for calcium gave an average of 12.63 mgm. per 100 cc. of serum for 55 analyses, with a range of 9.96 to 16.18 mgm. The results indicate no noticeable differences in the calcium content of young and mature animals. The average for 5 cows from four to nine years of age is 13.31 mgm., while that for 9 calves ranging from one to twenty-eight days of age is 13.22 mgm. Our results are quite definitely higher than those of all the other investigators except Hart and his co-workers (16).

Carbon dioxide. In our work we have determined carbon dioxide on 36 individuals with results ranging from 31.5 to 76.8 cc. per 100 cc. of plasma. The average value is 58.93 cc. A noticeable observation is that the plasma of cows' blood binds less carbon dioxide than that of calves. This has also been noted by Blatherwick (6). Males give a higher average than females. Our

average value is very close to that found by Robinson and Huffman (23).

SUMMARY

Fifty-nine samples of blood from dairy calves and cows have been analyzed for hemoglobin, non-protein nitrogen, urea nitrogen, uric acid, creatine, creatinine, sugar, chlorides, phosphorus, calcium and carbon dioxide binding capacity. The results are given in tables 1 and 2.

The authors wish to acknowledge the coöperation of Dr. S. I. Bechdel of the Department of Dairy Husbandry in allowing us free access to the dairy herd.

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BOOK REVIEWS

Milk and Milk Products. CLARENCE HENRY ECKLES, WILLES BARNES
COMBS, AND HAROLD MACY, University of Minnesota. Published
by McGraw-Hill Book Company, New York, 1929.

This book was prepared to serve as a text for the first course in dairy
manufactures in colleges of agriculture. It is based upon the experi-
ences of the authors in teaching this course. Their accomplishments in
teaching will recommend this book to everyone concerned with the
teaching of the introductory course in dairying.

The book covers the composition and properties of milk, a brief survey
of bacteriology, methods of testing, the processing of market milk, and
the manufacture of butter, cheese, ice cream, dry milk, condensed milk,
and milk by-products.

The introduction, giving the history and development of the dairy
industry, is especially interesting even to an advanced student. The
authors are to be commended for having omitted the customary chapter
on the physiology of milk secretion and the over-used diagramatic sec-
tion of a cow's udder. The organization of material is excellent except
that the appendix contains tests even though it is preceded by a chapter
on "Miscellaneous Tests." There is some repetition of tests.

It is doubtless essential to make statements in introductory books
without all the necessary qualifications. One of the purposes of ad-
vanced courses is to explain why the definite, simple statements made in
the elementary course are not absolutely true. In this book one might
desire more detail concerning certain statements, as, for example the
"slight practical significance" of the germicidal action of milk in view of
its extensive use in not cooling morning's milk; the delivery of 18 grams
by the 17.6 cc. pipette, etc. Such points are minor except to illustrate
the difficulty encountered in preparing an introductory book which must
give positive statements with little explanation, and the need for toler-
ance in interpreting every word and phrase with absolute exactness.

In these days when plant managers, advertising specialists, econo-
mists, etc., enter the dairy industry without an intimate knowledge of
the industry, this book ought to make a special appeal to them. The
information would be as valuable and available to them as it is to the
college student.

<div align="right">A. C. DAHLBERG.</div>

NORMAL VARIATIONS IN THE CALCIUM CONTENT OF THE BLOOD OF DAIRY CATTLE*

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In a previous paper (1) we have shown the extent to which the inorganic phosphate of the blood plasma of dairy cattle may vary from day to day. A biometric study of 60 sets of 3 consecutive-day analyses, taken at monthly intervals, revealed the fact that 76 per cent of the samples varied significantly from the theoretical values indicated by the coefficients of correlation of the respective days. Although the mean deviation of all actual values from the theoretical was only 14.3 per cent, several deviations exceeded 50 per cent and one reached 123 per cent of the expected.

Although the blood samples which furnished these data were taken primarily for the purpose of studying the phosphate concentration in the blood, the calcium content of the plasma was also determined. The data thus secured furnish the basis for the present report.

The literature to-date discloses the calcium content of about 350 samples of blood plasma or serum from presumably normal cattle (2–13) and over 100 samples from cattle suffering from milk fever (11–15). The great majority of the normal samples show a range of 9.0 to 12.0 mgm. per cent calcium with extreme values of 4.0 (8)[1] and 24.4 (5)[2] mgm. per cent, respectively. The lowest value so far reported (13) is 2.7 mgm. per cent for a cow "down" with milk fever. A great variety of dietary, physiological, environmental, and other conditions are represented among the animals that furnished the presumably normal samples.

[1] From a calf on a diet of whole milk and paraffin oil.

[2] From a lactating cow on a ration of timothy hay, corn silage, and grain mixture. The figure is comparable to the calcium content of whole blood in dairy cattle, but not to that in serum or plasma.

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It is difficult to determine from this mass of data what normal variations may be expected to occur when the dietary and physiological conditions are presumably constant for an individual animal. It has been the practice, for the most part, to interpret the effects of dietary and other changes on the basis either of a single sample or the average of several single samples taken at arbitrary intervals. Robinson and Huffman (7) have attempted to determine the daily variation in the calcium of the blood of 4 cows on a uniform diet in a series of successive daily samples ranging from 5 to 8 days. They encountered one twenty-four hour fluctuation of 2 mgm. per cent in one eight-day series whose mean calcium was about 8.5 mgm. per 100 cc. of plasma. Their data, however, are too meagre for biometric analysis, although they are the only data so far published on blood calcium of cattle which deal with the variations under uniform physiological conditions.

Meigs, Blatherwick, and Cary (3) concluded from a study of 41 calcium determinations made by them that, "the concentration of calcium in the plasma of cows is quite constant. Small variations can be induced by varying the amount supplied with the rations, but the chief controlling factor is probably the concentration of bicarbonate in the plasma.[3] It is probable that the concentration of calcium tends to vary inversely with that of the bicarbonate." This explanation of the variations in plasma calcium was accepted by Hart and associates (5), but there seems to be little support for it in the data secured by Robinson and Huffman. The largest day-to-day fluctuations in calcium encountered in the short series of the latter investigators were not accompanied by any appreciable change in carbon dioxide. Conversely they record great changes in carbon dioxide without any corresponding change in calcium. Likewise there is no indication that calcium and carbon dioxide fluctuate inversely.

Salvesen and Linder (16) have demonstrated that changes in serum protein in human nephritis are paralleled by changes in

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[3] The probable relation of calcium to bicarbonate concentration in blood plasma appears to have been pointed out first by Rona and Takahashi in 1913 (Biochem. Z., xlix, 371–380).

serum calcium, but that in experimental tetany in dogs following parathyroidectomy, the drop in serum calcium is not accompanied by any change in serum protein (17). Hastings, Murray, and Sendroy (18) believe that the formula

$$[CA] = 0\ 014\ [P] - 1.4$$

expresses the relationship between calcium and protein in blood serums, the calcium being in mM. per kilogram H_2O and protein in grams per kilogram H_2O. Peters and Eiserson (19) have confirmed the direct relationship between serum protein and serum calcium in human nephritis. On the basis of their results and the generally accepted idea that the concentration of calcium in serum is likewise dependent (inversely) upon the level of inorganic phosphate, Peters and Eiserson propose the formula

$$Ca = -0.225\ P + 0.556\ protein + 7[4]$$

to express the relation between serum calcium and what they regard as the two principal controlling variants in the blood. However, the errors which may be involved in applying this formula may exceed 2 mgm. Whether similar approximate relations hold true for cattle blood remains to be determined.

EXPERIMENTAL

The data which we present at this time are a biometric study of 60 sets of blood samples, each representing 3 consecutive days, taken at monthly intervals from August to December, 1926. The animals used were all in the University dairy herd. Twenty-four different animals, in all, furnished the data. Five animals had only 1 period of 3 consecutive days, 10 animals had 2 periods, 1 animal had 3 periods, and 8 animals had 4 periods. The ages of the animals ranged from calves to mature cows, some of the latter being in milk. The diets used represented various planes of calcium and phosphorus intake.

The blood was drawn from the jugular vein at the same hour each day. It was allowed to flow into 100-cc. glass tubes con-

[4] In this formula Ca and P are milligrams per cent and protein is per cent concentration.

TABLE 1

Day-to-day variations in calcium in successive three-day periods per 100 cc. plasma

ANIMAL	PERIOD 1 Ca₁ a	Ca₁ b	Ca₂ a	Ca₂ b	Ca₃ a	Ca₃ b	PERIOD 2 Ca₁ a	Ca₁ b	Ca₂ a	Ca₂ b	Ca₃ a	Ca₃ b	PERIOD 3 Ca₁ a	Ca₁ b	Ca₂ a	Ca₂ b	Ca₃ a	Ca₃ b	PERIOD 4 Ca₁ a	Ca₁ b	Ca₂ a	Ca₂ b	Ca₃ a	Ca₃ b
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
E 33	13.17		11.30	11.57	12.01	12 56	15.77	16.15	13 11	13 30	11.21	11 21	12.35	12 35	12.92	12 35	12.73	12 92	13 02		13.11	13.20	12.35	12 54
E 73	13.17	12.28	12.46	13.70	11.57	11 39	13.91	14.63	12.44	12 64	13.49	13 11	11 13	30	12.92	13	11 97	12 16	11 97	12 16	12.35	12.35	12.35	12.35
E 74	10.50	10.50	10.86	10.86	10.14	11.03	11.78	11 78	11 59	11 59	12.16	11.78	11 78	11 97	11 10	11 01	11 97	12 16	10.45	10.54	11.40	11.01	9.88	9.88
E 75	12.46	12.46	11.92	11.92	11.75	12.64	14.92	14.92	14 25	14.44	14.63	14.63	12 92	13 11	12 73	12 92	13.87	14 25	13.20	13.11	12.73	13	11.78	11.78
E 91	11.83	11.66	11.57	11.75	11.92	12.28	12.92	13.11	11 92	12 35	12.92	12.92	10.83	11.40	11.40	11.59	13 11	13 11	11.21	11.11	11.97	11.97	11.59	11.78
E 92	14.10	13.72	11.28	11.28	12.78	12.78	12.54	12.54	13 30	12 73	12.73	13	11.21	11.40	12.92	13	11.97	11.97	11.11	11.11	10.45	10.45	9.88	10.07
E 93	13.16	13.34	12.03	12.22	11.09	10.53	14.44	14.44	13 30	13	13.68	13	17.10	17.10	13 11	13 11	15.96	16.15	13.30		12.92	13.11	13.20	13.20
E 94	12.78	13.34	13.16	13.16	12 60	12 97	15.58	15.77	13 30	13 30	15.58	16.15	17.10	40	14 92	15 20	15.96	16.15	11.40	11.59	11.40	11.49	9.97	9.97
E 84	15.48	14.95	14.24	15.12	14.59	14.77	12.35	12.35	11 97	11.97	16.72	16.15	11.11	11 40	9.88	9.97	9.97	9.97						
E 58	11.39	11.80	11.12		11.30	11 48	14.06	13.87	12 92	12 92	12.54	12.35												
E 68	15.04	15.04	13.63	13.16	13.66	11.84	11.59	11.59	10 92	10.92	10.83	10.83												
E 77	13.72	13.91	14.10	14.10	13.16	13.16	11.21	11.40	11 97	11.68	10.54	10.83												
E 80	15.94	16.55	16.37	16.55	14.68	14.50	14.25	14.25	13 68	13	13.68	13.68												
E 81	16.37	17.08	16.55	16.20	15.66	14.95	13.68	13.68	11 01	11	14.63	14.92												
E 83	13.35	13.53	12.46	11.92	12.46	12.10	11.97	12.10	11 49	11.49	11.78	11.97												
E 85	17.26	17.46	16.02	15.66	13.35	13.17	14.25	14.25	12 73	12.92	12.92	12.82												
E 86	15.04	15.60	12.78	13.16	12.22	12.22	11.01	11.01	10 45	10.45	10.07	10.16												
E 88	14.59	14.95	13.35	13.53	17.08	17.62	11.97	12.35	11 01	11.01	14.92	14 63												
E 89	11.28	11.28	11.84	12.22	13.16	13.34	12.16		10.26	10.26	10.26	10.45												
E 78	16.02	16.62	14.25	13.87	12.73	13.11																		
E 87	13.91	13.91	11.28	11.84	12.60	12.78																		
E 95	11.97	11.97	12.54	12.54	14 92	15.39																		
E 96	12.16	12.54	11.78	11.97	12.54	12.54																		

taining 1 cc. of a saturated solution of sodium citrate.   A 50-cc. sample was drawn from each animal at each bleeding.   The blood in each tube was mixed thoroughly with the sodium citrate solution immediately to prevent coagulation.   It was then centrifuged to separate the corpuscles from the plasma.   Sufficient plasma was pipetted off at once and preserved in a refrigerator until analyzed.   In nearly all cases the analysis was begun the same day the sample was drawn.

The method of analysis employed throughout was the Clark and Collip (20) modification of the Kramer-Tisdall procedure. Duplicate analyses were made of all samples.

The complete data, including the duplicate analyses are given in table 1.   In this table Ca is the milligrams of calcium per 100 cc. of plasma, the subscripts 1, 2, and 3 referring to the first, second, and third days, and the subscripts a and b to the duplicate analyses.

The coefficients of correlation of the respective days, calculated by the formula of Harris (21) are as follows:

$$r_{Ca_1 Ca_2} = +0\ 762340 \pm 0\ 036471$$
$$r_{Ca_1 Ca_3} = +0\ 624557 \pm 0.053111$$
$$r_{Ca_2 Ca_3} = +0\ 627991 \pm 0\ 052737$$

It is apparent that the coefficients of correlation of the values obtained on successive days are fairly high when the data are considered as a whole.   The coefficients, however, are considerably lower than for the inorganic phosphate of the same samples, as shown in our previous paper (1), where the coefficients ranged between +0.93 and +0.97.   Inspection of the data in the table shows that there were marked variations from day to day.   Using the same method for showing the percentage deviation of the actual values from the theoretical as was employed for inorganic phosphate in the previous paper (1), the following equations were derived for the calculation of these deviations.

$$Ca_2 = +2\ 459 +0.762\ Ca_1$$
$$Ca_3 = +4\ 432 +0\ 625\ Ca_1$$
$$Ca_3 = +4.908 +0.628\ Ca_2$$

The accompanying figure shows the percentage variations of the actual values from the theoretical values obtained from the above

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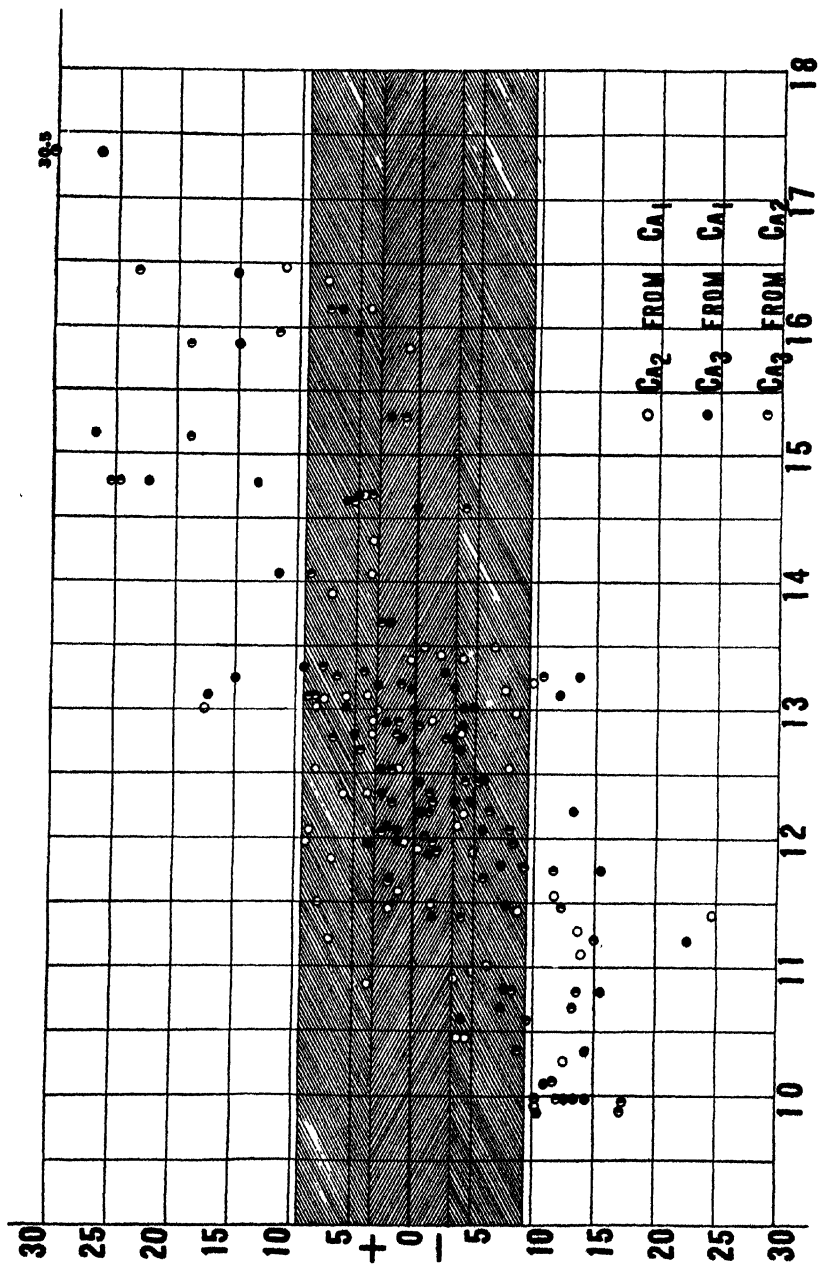
FIG. 1

equations.   The mean value of these figures is 12.73 mgm. for the calcium and 7.3 per cent for the deviation.   The physiological significance of the individual variations is revealed by the points within and outside of the cross-hatch lines.   The lines drawn at $+9.5$ and -9.5 per cent each represent the maximum numerical percentage difference that occurred in any of the duplicate analyses.   All the points outside of these lines are therefore physiologically significant.   The lines drawn at $+3.25$ and -3.25 per cent, respectively, were chosen arbitrarily because calculation of the analytical differences between duplicate samples showed that 86 per cent of the mean values of duplicate analyses had an error of 3.25 per cent or less.   This means that 86 per cent of the points between the 3.25 per cent and the 9.5 per cent lines represent physiologically significant variations.   In like manner 14 per cent of the points between the 0 line and the 3.25 per cent lines are significant.   A summation of the significant variations of the actual from the theoretical values shows a total of 125 out of 180 or 69.4 per cent.

DISCUSSION OF RESULTS

As in the case of the inorganic phosphate, the mean value for blood calcium in dairy cattle cannot be determined with accuracy by random sampling.   Significant fluctuations occur from day to day, not at present explainable, even when the samples are taken under presumably identical physiological conditions.   Although there are fairly high coefficients of correlation of the consecutive samples in our data, yet nearly 30 per cent of the individual samples varied 10 per cent or more from the regression line. This represents a mean actual variation of over 1.25 mgm. of calcium per 100 cc. of blood plasma.   The maximum actual variation encountered was 4.05 mgm. calcium per 100 cc. plasma.

The distribution of the points on the chart is not without significance.   The similar chart for inorganic phosphate, given in our previous paper (1) shows no relation between the actual phosphate value and the direction of the deviations from the theoretical. In the case of calcium, however, it is evident that a relatively low concentration of calcium on the initial day of a three-day series

definitely predicts that the subsequent days' concentration will be *lower* than is demanded by the coefficient of correlation, whereas a relatively high concentration of calcium on the initial day of a three-day series definitely predicts that the subsequent days' concentration will be *higher* than is indicated by the coefficient of correlation. No logical explanation for this phenomenon has occurred to us. The distribution of the points on the chart is too definite, however, to be ignored. It is especially remarkable because the coefficient of correlation of the blood calcium on successive days is definitely positive.

CONCLUSIONS

The calcium content of the blood plasma of dairy cattle is subject to significant fluctuations of an undetermined cause, in spite of a high coefficient of correlation of the calcium content of the plasma on successive days.

When a three-day series of blood samples is secured from dairy cattle on successive days, any deviation of the plasma calcium from the theoretical value will be negative when the plasma calcium is relatively low, and will be positive when the plasma calcium is relatively high.

ACKNOWLEDGMENTS

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THE BIOMETRY OF CALCIUM AND INORGANIC PHOSPHORUS IN THE BLOOD PLASMA OF DAIRY CATTLE

APPLICATION OF RESULTS TO BONE MINERALIZATION[*]

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The problem of the nature of the calcium compounds of blood has long attracted the interest of biochemists, physiologists, and clinicians. The problem has many applications to both normal and pathological conditions.

Rona and Takahashi (1) concluded that calcium occurs in blood serum in two forms only, as calcium bicarbonate which is completely diffusible, and as a calcium-protein compound, the calcium of which is non-diffusible. The possibility of calcium occurring in the blood as calcium phosphate was also considered by Rona and Takahashi but rejected on the following grounds. They pointed out that the only form of calcium phosphate that could exist at the hydrogen ion concentration of blood would be an insoluble one. They reasoned that this compound would therefore have to be present in suspension and would thus be non-diffusible, whereas they found that the inorganic phosphorus of the serum is entirely diffusible.

According to Rona and Takahashi the calcium bicarbonate concentration of blood is expressed by the relation

$$\frac{(Ca'') \ (HCO_3')}{(H')} = 350 \times 10^{-8}$$

From this it appears that at constant pH the calcium concentration of the blood varies, in part at least, inversely with the bicarbonate concentration.

The solubility of calcium carbonate in blood has been considered extensively by Hastings, Murray, and Sendroy (2) and by Sendroy and Hastings (3) both from an experimental and from a thermodynamic standpoint. Their conclusion is that blood is not supersaturated with respect to calcum carbonate; it is probably undersaturated. Blood serum, in their studies, showed a stoichiometric solubility product for calcium carbonate of $10^{-6.40}$ compared with $10^{-7.40}$ for calcium carbonate in salt solutions of comparable ionic strength.

The relationship between calcium and protein in blood has been investigated by Salvesen and Linder (4), Marrack and Thacker (5), Loeb (6) (7), Loeb and Nichols (8) (9), Hastings, Murray, and Sendroy (2), and Peters and Eiserson (10). All of these studies support such a relationship. Salvesen and Linder found that decreases in serum calcium in non-uremic cases of Bright's disease without phosphate retention are paralleled by decreases in plasma protein. Marrack and Thacker investigated the dialysis of calcium from solutions of blood proteins and from blood serum and concluded that the calcium of body fluids is partly in the form of non-ionized protein compound, the formation of which accounts for the non-diffusible calcium of serum. Loeb, and Loeb and Nichols have studied the diffusibility of calcium from solutions of egg albumin, from serum globulin and from serum itself from the standpoint of the Donnan equilibrium theory and have concluded that essentially all the calcium is accounted for as ionized and non-ionized calcium-protein compounds. Hastings, Murray, and Sendroy have formulated the the equation, [Ca] = 0.014 [P] − 1.4[1] to express the relation between the total calcium and protein concentration in serum. The equation was based on their own data and the data of Salvesen and Linder. Peters and Eiserson have modified this equation to include the inorganic phosphorus of the blood. Their equation is Ca = −0.255 P + 0.556 protein + 7[2]. It is not

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[1] In this equation [Ca] = mM calcium per kilogram H_2O and [P] = gram protein per kilogram H_2O. The minus sign is an error according to Peters and Eiserson (10).

[2] In this equation the Ca and P are milligram per cent and protein is per cent.

surprising that attempts made by Peters and Eiserson to calculate the calcum of blood by means of this equaton led in some instances to errors of great magnitude.   Casual inspection shows that the expression they have formulated has no biological significance when the calcium equals 7 mgm. per cent, because at this value the equation resolves itself into P = 0.218 protein. This relation is, to say the least, improbable.   In fact the alignment chart given by Peters and Eiserson shows no such relation between blood phosphate and blood protein when calcium equals 7 mgm. per cent.

We have applied the Peters and Eiserson formula to several samples of normal cattle plasma with the results shown in table 1.   The data show that the formula has no value for cattle blood.

TABLE 1

Deviation of calculated from observed blood plasma calcium in dairy cattle, using the formula of Peters and Eiserson

COW	CALCIUM FOUND	CALCIUM CALCULATED	DEVIATION OF CALCULATED FROM ACTUAL VALUE	
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*per cent*
66	7.70	8.99	+1.29	+16.8
82	9.35	9.40	+0.05	+ 0.5
E33	8 09	9.56	+1.47	+18.2
87	9.60	9.41	−0.19	− 2.0
60	7.70	10.14	+2.42	+31.4
21	10.23	9.95	+0.28	− 2.7

The samples were picked because they showed considerable range of calcium.

Although, as already stated, Rona and Takahashi rejected the probability of calcium existing in the blood as phosphate, there is indirect evidence which is generally accepted as indicating a close relationship between calcium and inorganic phosphate in the blood.   Binger (11), and Tisdall (12) showed that the injection of phosphates reduces blood calcium and causes tetany.   It should be pointed out, however, that although the results of Binger were attributed to the effect of the phosphate ion on blood calcium, Tisdall's results were interpreted as due to a changed sodium-calcium ratio.   Disodium phosphate was injected in

Tisdall's experiments.   In addition to this indirect evidence there exist the extensive thermodynamic studies of Holt, La Mer, and Chown (13), Holt (14), and Sendroy and Hastings (3) (15), on the solubility of calcium phosphates in biological fluids. The former group of investigators conclude ,that, "serum is normally *supersaturated* with tertiary calcium phosphate to the extent of more than 200 per cent."   The experiments of the latter group of workers also support a condition of supersaturation of tri-calcium phosphate in blood.   On the other hand, Shear and Kramer (16), and Shear, Washburn, and Kramer (17) hold that the calcium phosphate of serum is di-calcium phosphate and that normal serum is either very slightly *undersaturated* or just saturated with respect to this compound.

The occurrence of calcium phosphate in blood in the form of $CaHPO_4$ or $Ca_3(PO_4)_2$ is generally regarded as necessary in order to account for the mineralization of bone.   Even though compounds of this character account for only a portion of the total calcium of the blood, it would appear probable that a large part of the inorganic phosphate would be involved.   In this case definite biometric relations should be found to exist between the calcium and inorganic phosphate of the plasma or serum.

The biometry of calcium and inorganic phosphorus in the blood serum of rabbits has been studied by Harnes (18) (19).   In a series of 80 samples, representing 80 animals received at the laboratory in groups of 10 during 8 consecutive months from October to May, the coefficient of correlation of $P \cdot Ca$ was

$$r_{P \cdot Ca} = -0.146 \pm 0.073$$

In a second series of 170 samples of the blood from 10 rabbits taken at weekly intervals for 17 weeks from October to May, the animals being kept under laboratory conditions, the coefficient of correlation of $P \cdot Ca$ was

$$r_{P \ Ca} = -0.124 \pm 0.051$$

In neither series is the result mathematically significant, inasmuch as the correlation in each case is less than three times the probable error.   It is of interest to note, however, that the corre-

lation is a negative one, thereby lending some support to the fact that there is frequently a tendency for the inorganic phosphate to vary inversely with the calcium in blood serum or plasma.

EXPERIMENTAL

The normal variations in the calcium and inorganic phosphate of the blood plasma of dairy cattle, secured at this laboratory, have

TABLE 2

Mean, standard deviation, and coefficient of variation of calcium and inorganic phosphate in blood plasma of dairy cattle

	MEAN VALUE	STANDARD DEVIATION	COEFFICIENT OF VARIATION	NUMBER OF SAMPLES
	mgm.	*mgm.*	*per cent*	
Ca_1[1]	13.25	1.81	13.67	60
Ca_2	12.56	1.46	11.61	60
Ca_3	12.76	1.71	13.44	60
Ca	12.86	1.69	13.17	180
P_1	4.94	2.85	57.77	60
P_2	4.75	2.52	53.09	60
P_3	4.85	2.72	55.98	60
P	4 85	2 70	55.74	180

[1] The subscripts refer to the day in the three-day series.

TABLE 3

Coefficient of correlation and probable error of calcium and inorganic phosphate in blood plasma of dairy cattle

	COEFFICIENT OF CORRELATION	PROBABLE ERROR	NUMBER OF SAMPLES
$^rCa_1Ca_2$	+0.762	±0.036	60
$^rCa_2Ca_3$	+0.625	±0.053	60
$^rCa_1Ca_3$	+0.628	±0.053	60
$^rCa\ Ca$	+0.628	±0.031	180
$^rP_1P_2$	+0.970	±0.005	60
$^rP_2P_3$	+0.954	±0.008	60
$^rP_1P_3$	+0.931	±0.011	60
$^rP\ P$	+0.946	±0.005	180

recently been published (20) (21). The data were secured from samples of blood taken on 3 consecutive days at monthly intervals. Twenty-four different animals furnished the blood. Five of these had only 1 period of 3 consecutive days, 10 animals had 2 periods,

1 animal had 3 periods, and 8 animals had 4 periods. There were, therefore, 60 sets of three-day samples.

The previous papers give the complete data for the calcium and inorganic phosphate for the consecutive days. The mean values, standard deviations, and coefficients of variation are given in table 2. The coefficients of correlation, calculated by the method of Harris (22), and their probable errors are given in table 3.

The original data also presented an opportunity to study the coefficients of correlation of the calcium and inorganic phosphate, and thus obtain further light on the probable importance of calcium phosphate in the blood. The results of these calculations are as follows:

$$r_{Ca_1P_1} = +0.116 \pm 0.086 \quad (n = 60)$$
$$r_{Ca_2P_2} = -0.109 + 0.086 \quad (n = 60)$$
$$r_{Ca_3P_3} = +0.008 + 0.087 \quad (n = 60)$$
$$r_{CaP} = +0.022 \pm 0.050 \quad (n = 180)$$

These results show even less correlation between calcium and inorganic phosphate in the blood plasma of dairy cattle than was found for rabbits by Harnes (18) (19). In the rabbit blood the coefficients did approach but did not reach mathematical significance, i.e., three times the probable error. In our calculations the summation correlation of $Ca \cdot P$ shows a probable error of nearly two and one-half times the correlation. The only conclusion that can be drawn from such a result is that the amount of calcium phosphate in the blood, at least of dairy cattle, is truly insignificant in relation to the other compounds of calcium and inorganic phosphate. We thus have mathematical proof for the conclusion of Rona and Takehashi (1) and of Loeb (6) (7), and Loeb and Nichols (8) (9) in so far as the occurrence of compounds of calcium and phosphate in the blood are concerned. It thus seems necessary to seek for an explanation of the mineralization of bone on other biochemical grounds than a mere precipitation of calcium phosphate from body fluid. Freudenberg and György (23) have proposed a theory which appears to deserve more recogniton that it has received. According to this theory

calcification of bone consists of the following stages: (a) A calcium-protein compound is formed between the collagen of the cartilage and the diffusible calcium ions from the blood; (b) HPO_4'' and HCO_3' ions from the blood react with the calcium bound by the collagen; (c) $Ca_3(PO_4)_2$ and $CaCO_3$ split off, releasing the calcium binding groups of the protein, so that the process may be repeated.

SUMMARY AND CONCLUSIONS

A high coefficient of correlation exists between the phosphate of the blood plasma of dairy cattle on successive days.

A fairly high coefficient of correlation exists between the calcium of the blood plasma of dairy cattle on successive days.

No correlation whatever exists between the calcium and inorganic phosphate in the blood plasma of dairy cattle.

No biologically significant amount of calcium phosphate occurs in the blood.

The mineralization of bone is not explainable on the basis of a mere precipitation of bone salts from body fluid.

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EFFECT OF THE ELECTROPURE PROCESS AND OF THE HOLDING METHOD OF TREATING MILK UPON BACTERIAL ENDOSPORES*

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The Electropure process of milk "purification" has attracted some attention in recent years. The theory and mechanics of the Electropure process have been thoroughly described in the literature, and will, therefore, not be discussed in detail in this paper. Briefly, the process consists of passing a column, or rather a continuous stream of milk, through a narrow gauge rectangular chamber, the side walls of which consist of two especially prepared carbon electrodes. The resistance offered by the various electrolytes in the milk to the passage of electricity immediately causes the liberation of heat sufficient to effect a reduction of approximately 99 per cent in the bacterial count of the milk without apparently altering the quality of the milk.

During the course of some investigations carried on for the purpose of studying the types of microörganisms in raw market milk which seemed persistently to resist the Electropure process of milk "purification," it was oberved that strikingly few, if any, of the organisms which survived the process were spore-formers. As a result of this observation, series of tests were conducted in order to determine definitely whether or not the Electropure process actually destroyed spores, and, if so, to what extent.

In 1924 Carpenter et al (1) working with a culture of *Bacillus subtilis*, a microscopic examination of which revealed that the majority of the cells had developed spores, obtained approximately an 80 per cent reduction when 45 to 50 gallons of inoculated milk were subjected to electrical pasteurization.

The organisms used in our tests were all obtained from the stock cultures of the Michigan State College bacteriological

laboratories. The cultures were grown for four to six weeks on agar slants at room temperature, and before using each culture was examined microscopically to determine the approximate extent of sporulation. All cultures showed from 80 to 90 per cent spores when used. Those cultures which were too dry to be put into suspension by the usual method of washing with saline solution, were ground in sterile mortars with sterile sand, then washed in salt solution and filtered through paper. Pure cultures of each organism were subjected both to the holding method of pasteurization and to the Electropure process according to the procedure outlined below.

The holding method was simulated as follows: 10 cc. lots of skimmed milk were placed in test tubes and rendered sterile by autoclaving at 10 pounds pressure for 20 minutes. Platings were made to check the sterility of the milk. Skimmed milk was used to avoid any interference from the fat layer in the test tubes. Each tube was then inoculated with about 0.1 cc. of the particular spore suspension to be tested. Immediately following inoculation, plate counts were made of each tube on standard agar plates using dilutions of 1:1000, 1:10,000, and 1:100,000. The tubes were then placed in an automatically controlled water bath at 62.8°C. and held at that temperature for 30 minutes from the time the milk in the tubes reached the desired temperature. At the end of the heating period the tubes were immediately transferred to an ice water bath and cooled to 10°C. Plate counts were then made of each tube, and the percentage reduction as a result of the treatment, was determined.

Each culture used in the above tests was then individually subjected to the Electropure process according to the following procedure. A two liter sample of skimmed milk previously rendered sterile by autoclaving for 20 minutes at 10 pounds pressure was heavily inoculated with saline suspension of spores (pure culture) of the organism to be tested. The milk used was carefully checked for sterility by running control plates. The flasks of milk were inoculated with such quantities of suspension that 1 cc. of the milk gave a plate count of 50,000 or over before treatment. After thoroughly shaking to insure an even distribution of

organisms, the inoculated milk was plated according to standard methods of milk analysis in order to obtain accurate initial counts. The milk was then subjected to the Electropure treatment at a temperature of 71°C. The milk entered the machine at room temperature and reached the desired temperature by the time it had travelled two-thirds of the way through the chamber. Thirty-five to forty seconds were required for the milk to pass through the machine, during 10 to 14 of which it was subjected

TABLE 1

Percentage reduction in spore count due to pasteurization

CULTURE	HOLDING PROCESS (62.8°C.—30 MINUTES)			ELECTROPURE PROCESS (71°C.)		
	Initial count	Final count	Percentage reduction	Initial count	Final count	Percentage reduction
First trial						
B. anthracis.........	2,170,000	2,110,000	2.7	2,400,000	4,200	99.5
B. megatherium....	4,810,000	4,812,000	0	2,600,000	740,000	71.5
B. subtilis..........	490,000	550,000	0	2,100,000	360,000	82 0
B. mycoides.........	678,000	682,000	0	3,000,000	5,000	99 9
B. mesentericus.....	1,162,000	1,140,000	1.7	7,800,000	150,000	98 0
Second trial						
B. anthracis.........	947,000	944,000	0 3	1,850,000	4,700	99.7
B. megatherium.....	3,720,000	3,780,000	0	14,500,000	570,000	90.6
B. subtilis..........	1,970,000	1,960,000	0.5	1,140,000	13,000	98.0
B. mycoides.........	1,620,000	1,400,000	13 0	4,200,000	2,000	99.9
B. mesentericus.....	2,180,000	2,160,000	0.9	5,200,000	310,000	94.0

to a temperature of 71°C. A sample was drawn in a sterile flask (about 75 to 100 cc.) after the rate of flow and temperature had been properly adjusted and were constant. Immediately upon drawing, the sample was cooled to below 20°C. and plated as above. The results of these tests (plate counts and percentage reduction as a result of treatment for each process) are shown in table 1.

SUMMARY

Series of laboratory tests were conducted with some common spore-forming organisms in order to compare their relative re-

sistance to the Electropure and holding methods of pasteurization. The cultures were from six to eight weeks old when used, and microscopic examinations showed them to contain from 80 to 90 per cent spores. The results obtained in these tests, as shown in table 1 are markedly in favor of the Electropure process. Particular attention is drawn to those results obtained with *Bacillus anthracis* which is known to be an extremely resistant organism. The percentage reduction in the case of this organism ranged from 0.3 to 2.7 in the holding method, and from 99.5 to 99.7 in the Electropure process. The results of the tests with *B. subtilis, B. mycoides, B. mesentericus,* and *B. megatherium* are equally striking.

Concluding from the above results concerning spore destruction the Electropure process at 71°C. with momentary holding proved to be superior to pasteurization at 62.8°C. for 30 minutes, in these laboratory tests.

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MEASURING THE INFLUENCE OF HORMONE AND MECHANICAL STIMULATION BY MEANS OF A NON-FECUND LACTATION*

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Milk secretion is caused by two factors (1), namely, an unusually marked internal or hormone stimulus, and an external mechanical or nervous stimulation produced by the act of milking. The latter has less influence than the former. Lane-Claypon and Starling (2) established a relationship between development of the foetus and of the mammary gland. Likewise, d'Errico (3) found that the blood of a pregnant bitch exerted an inhibitory influence on milk secretion when injected into the veins of a lactating bitch.

Pregnancy and parturition have a marked influence on the development and activity of the mammary gland, and provide the initial stimulus (1) for milk production, but are not essential (4) in connection with a limited milk yield. Literature relative to milk secretion by virgin and non-fecund females, and males of several species, has been reviewed extensively by Hammond (4), Hill (5), Marshall (6), Monvoisin (7) and Velich (8).

PRESENTATION OF DATA

An unusual instance of non-fecund lactation occurred with the Holstein-Friesian cow, Rosa Segis Johanna 446097, in the Oklahoma Agricultural Experiment Station dairy herd. This cow had previously completed yearly semi-official records of 18,375.7 pounds of milk, 3.45 per cent fat, 635.13 pounds of butterfat as a junior two-year-old, and of 24,313.7 pounds of milk, 3.55 per cent fat, 864.36 pounds of butterfat as a senior three-year-old. She

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was placed on semi-official test again, but was withdrawn from test after an operation to remove a nail that had pierced the reticulum and diaphragm near the heart. A subsequent operation was attempted on an infected Fallopian tube. Following the latter operation, her ovaries ceased to function, voice assumed a masculine tone, crest began to develop, coccygeal vertebrae showed an inclination to rise as in certain cases of sterility in cows, and she exhibited the desire to mount other cows in the herd at every opportunity. She went dry on August 17, 1925, and was excessively fat. Her blood was negative to the agglutination test for the *Bacillus abortus* of Bang (*Brucella abortus*).

Rectal examinations were made, and the ovaries massaged regularly by Dr. E. E. Harnden, veterinarian on the staff of the Oklahoma Agricultural and Mechanical College. A cyst on the right ovary was ruptured on January 14, 1926, and after the usual interval of four days thereafter the cow came into heat, but was not bred. She showed oestrum again at a three weeks interval on February 8, 1926, but was not deemed in condition to be bred.

Two hundred cubic centimeters of oestrual hormone solution were injected in 10 cc. doses between January 15 and February 11, 1926, without any effect being noted that could be attributed to the injections. The influence of this solution, according to Dr. H. S. Murphey (9), is that "The only known hormone action of the corpus luteum is to produce a substance which affects the peristalsis of the tubular part of the genitalia. You can find plenty of statements in the literature about this structure being a gland of internal secretion, but there is no proof which will stand experimental investigation."

It is not known whether the ovarian extracts exerted any influence, but the fact remains that the cow gradually resumed feminine characteristics of voice and behavior. She was irregular in the oestrus cycle. After being pronounced in good health by the veterinarian, she was bred on May 3 and 15, 1926, but did not conceive to either service. She was bred five more times at various intervals and finally conceived on July 11, 1927. A 92-pound bull calf was born on April 8, 1928 following a 273-day gestation period.

The most interesting fact concerning this cow, is that after being dry from August 17, 1925, and not pregnant, her udder began to develop in June, 1926. One quarter was found to be infected. In treating it, this quarter was milked out daily. Stimulation of the udder and teats brought the cow into milk. From July 1,
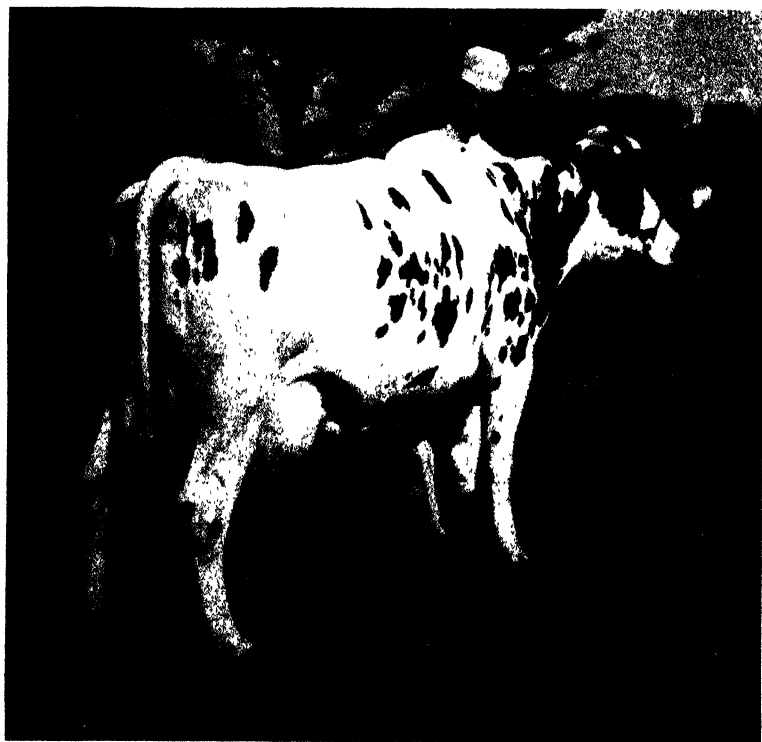
FIG. 1. ROSA SEGIS JOHANNA 446097 AS A FOUR YEAR OLD

1926 to August 3, 1927, she was milked twice daily. Since conception occurred only 24 days prior to completion of this non-fecund lactation, no decline in milk flow due to pregnancy was anticipated (4, 10, 11).

As a measure of the factors governing milk yield, the non-fecund lactation is compared with two previous and one subsequent lactations following normal gestation periods. Although the cow was
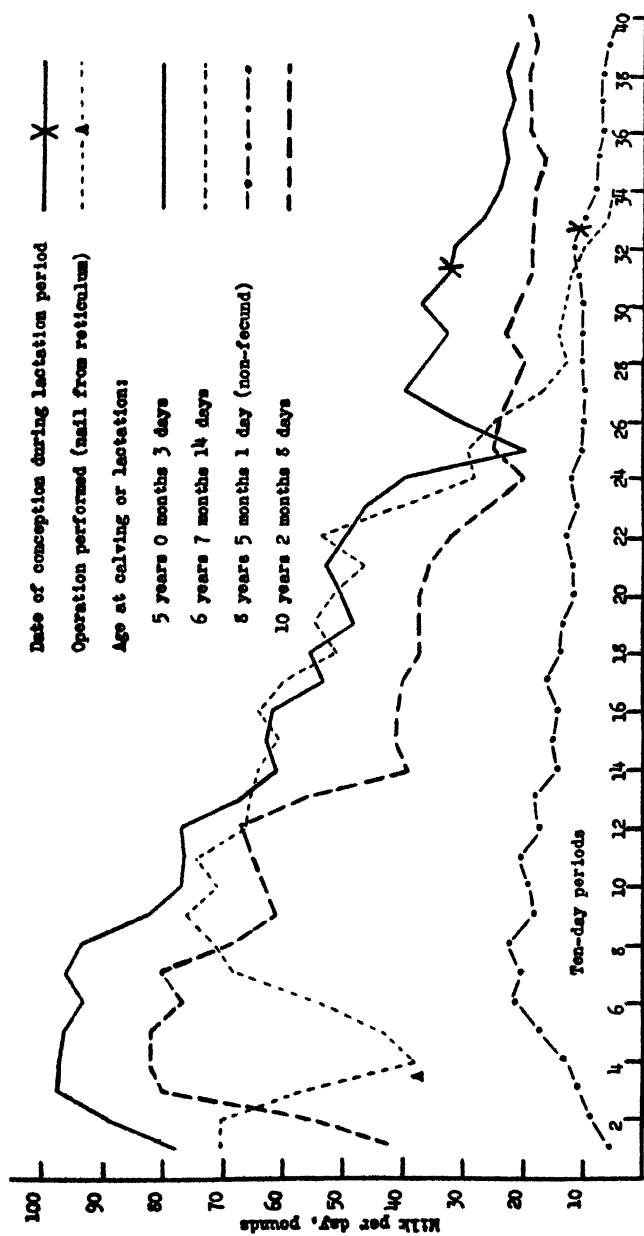
FIG. 2. NON-FECUND AND NORMAL LACTATION CURVES OF ROSA SEGIS JOHANNA 446097, COMPUTED BY TEN-DAY PERIODS, INCLUDING DATE OF CALVING AS THE FIRST DAY

milked twice daily during the non-fecund lactation, during the early parts of the three normal lactations she was milked three times daily in order to care for the high milk yields. Milk yields in the highest month of the normal lactations, were 2,987.5, 2,287.0 and 2,552.1 pounds respectively, as compared with 611.8 pounds in the highest month of the non-fecund lactation.

The lactation curves are tabulated by ten-day periods, including the day of calving as the first day. These are presented in figure 2. Production records, length of gestation periods, and age of the cow at time of calving, are presented in table 1. Feed and care were given the cow commensurate with her requirements for maintenance and production.

TABLE 1

Lactation and gestation records of Rosa Segis Johanna 446097

AGE AT CALVING			PRECEDING GESTATION	PREGNANT DURING LACTATION	LACTATION RECORDS			
					Length	Milk	Test	Fat
yrs.	mos.	days	days	days	days	pounds	per cent	pounds
2	1	21	*	*	365	18,375.7	3.45	635.13
3	9	27	*	*	365	24,313.7	3 55	864.36
5	0	3	274	115	423	21,612.6	3 66	790.97
6	7	14	282	0	338	15,014.6	3.99	599.65
8	5	1	0	24	399	5,199 1	3.41	177.16
10	2	8	273	0	523	17,024.8	3 30	562 11

* Prior to purchase by the Oklahoma Agricultural Experiment Station.

DISCUSSION OF RESULTS

Normal lactation curves measure the combined effect of the factors that govern milk production. Previously, we (12) showed maximum daily milk yield and persistency of lactation to be hereditary characters involved in total milk yield during normal lactations. The graphs in figure 2, show the effect of the mechanical and hormone influences on maximum daily milk yield, rate of decline in milk production, length of lactation, and time of conception of the mature Holstein-Friesian cow Rose Segis Johanna during three normal and one non-fecund lactations.

The normal lactation curves shown in the graph have the char-

acteristic maximum daily milk yield which measure the combined influences of the internal stimulus released at parturition, and of the external mechanical stimulus arising from the act of milking. The non-fecund lactation curve shows practically no influence of internal stimulus, but measures wholly the effect of the external mechanical stimulus upon milk flow. Gestation was not advanced sufficiently to exert an influence (4, 10, 11) on the two lactations involved.

When the normal and non-fecund lactation curves are superimposed, the *difference* between them measures largely the effect upon milk flow, of the internal stimulus resulting from gestation and released at parturition. Since the cow was milked three times daily to care for the extra milk yield in the three normal lactations, allowance must be made for the influence of the third milking daily. This has been variously estimated (13, 14, 15) as increasing the milk flow from 6.0 to 21.4 per cent under ordinary herd conditions, and as high as 48 to 72 per cent (10, 16, 17) under official testing conditions. (Official testing conditions involve a plane of nutrition above the cow's requirements, milking 3 or 4 times daily, housing in a box stall, and other items of management that permit maximum inherited capacity for production to be attained.)

It is seen from the super-imposed graphs, that the stimulus arising from reproduction had its greatest influence in the first few weeks after parturition. This internal secretion gradually diminished either in amount or effect until it appeared that the last few weeks of lactation were prolonged by external stimulation alone (10).

Observation of the shape of the curve of non-fecund lactation shows that in this instance maximum daily milk yield was attained later in the lactation period, and that at the peak it was only 21 to 28 per cent as high as in the normal lactations. Also the rate of decline in milk flow was less rapid, due to absence of a diminishing influence of an internal stimulus or hormone. Duration of the non-fecund lactation compared favorably with that of three normal lactations by the same cow.

SUMMARY

The review of literature and limited data obtained from an unusual case of non-fecund lactation in a Holstein-Friesian cow, indicate that the internal stimulus produced by pregnancy and released at parturition, was the largest factor controlling maximum daily milk yield. The more rapid decline in milk flow in normal lactations than in the non-fecund lactation, is attributed to a gradually diminishing internal or hormone influence arising from reproduction. Maximum daily milk yield was attained later in lactation when influenced mainly by external mechanical stimulation from the act of milking. This internal stimulus originating from reproduction, was not necessarily a factor in determining persistency of milk flow, as indicated by duration of the lactation period. At the peak of production, total daily milk yield following normal reproduction, was from $3\frac{1}{2}$ to 5 times as great as when milk flow was induced by mechanical stimulation alone. A small part of the difference is attributable to the fact that the cow was milked thrice daily in the normal lactations.

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THE CHEMICAL ANALYSIS OF BUTTER

The recognized value of the directions for operating the Babcock test and of the specifications for Babcock test glassware promulgated by the committee on this method for the American Dairy Science Association prompted an extension of this type of activity. The methods are considered primarily from the viewpoint of accuracy and standardization of testing procedures within the industry. The work will be of greatest value if those concerned with the use of these tests will express their views to the chairmen of the various committees so that methods can be adjusted to give most general satisfaction. The following report constitutes the second procedure recommended through the committees on chemical methods. The method is adapted to the complete analysis of butter in the laboratory rather than for a rapid churn test.

The two general methods of making a complete analysis of butter in a creamery are: the procedure of the Association of Official Agricultural Chemists (A. O. A. C.), which is used by the Federal Government and State Chemists; and the Kohman test, which is simpler of operation and which better satisfies the creamerymen.

At present, only a few butter manufacturing plants are regularly making either daily or weekly analyses of butter for milkfat, water, salt, and casein. Most creameries make churn tests for water and salt and the milkfat is obtained by difference, after making an allowance for casein. The time is at hand when all creameries ought to know the complete analysis of their butter and a sufficient number of complete analyses should be made to make certain that the milkfat content is correct as secured by difference. This practice is followed at the present time in the better class of plants.

Recently Clarke (1) reported that during July and the first ten days of August, 14.2 per cent of the butter in interstate shipments through Chicago was adulterated. Probably most of this butter was not willfully manufactured with a low milkfat or a

high moisture content.   Undoubtedly some of it was illegal butter just because of carelessness in not testing it or on account of poor testing equipment and methods.

Both the Kohman and A. O. A. C. methods have a high degree of accuracy, when properly executed.   A comparison of them was made by Guthrie (2).   Among a large number of data presented by him, the table (table 1) using Kohman's own figures,

TABLE 1

Differences in Duplicates

LOT NUMBER	A O A C TEST		KOHMAN TEST	
	Water	Milkfat	Water	Milkfat
	per cent	*per cent*	*per cent*	*per cent*
1	0.02	0.09	0.030	0 040
2	0 07	0.11	0.020	0.010
3	0.03	0.08	0.028	0 020
4	0 04	0.14	0.047	0 065
5	0.01	0.02	0.014	0 036
6	0 06	0.01	0.060	0.060
7	0.07	0 03	0 010	0.056
8	0.03	0.00	0 020	0.066
9	0.02	0 02	0.020	0 071
10	0.02	0 03	0.021	0 123
Average. ...	0.037	0 053	0 027	0 0547

In the water analyses the average difference between the duplicate samples was 0.010 per cent in favor of the Kohman method.   The milkfat determinations showed a preference in accuracy of 0.0017 per cent for the A. O. A. C. method.

and the two succeeding graphs indicate a close comparison of these two methods of analyzing butter.

In order to determine the accuracy of the modified Kohman method, when the weighings were made under a watch glass, a set of twelve portions was taken from a single sample.   Approximately 10 grams of butter was put in an aluminum beaker, after which it was immediately covered with a watch glass and the weight was taken.   The modified Kohman method was then followed.   The first reading of the milkfat in this series, as noted in graph 1, showed an average variation of 0.113 ± 0.007 per cent, with a range from 0.002 to 0.337 per cent.   The moisture in

this reading had an average variation of 0.031 ± 0.002 per cent, with a range from 0.001 to 0.087 per cent. These portions were dried for an additional fifteen minutes, and were then cooled and

First reading:

Water	16.568	16.638	16.650	16.633	16.586	16.653	16.613	16.607	16.624	16.620	16.655	16.634
Milkfat	80.166	80.328	80.337	80.333	80.503	80.410	80.475	80.366	80.331	80.454	80.492	80.290

Second reading:

Water	16.028	16.666	16.656	16.653	16.639	16.654	16.631	16.615	16.643	16.645	16.659	16.660
Milkfat	80.163	80.328	80.334	80.323	80.500	80.417	80.493	80.376	80.353	80.459	80.492	80.288

Per cent
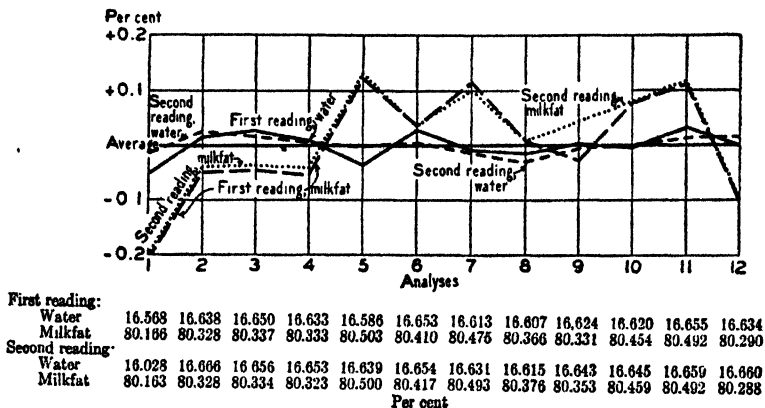
GRAPH 1. VARIATIONS WITHIN A SINGLE SAMPLE OF BUTTER

(Twelve analyses by the modified Kohman method)

Water	16 952	16.060	15.614	16 638	16.655	16.586	16.603	16.644	16.434	16.484	16.506	16.553
Milkfat	80.132	80.079	79.910	79.854	79.978	79.915	79.964	79.848	79.974	80.012	79.943	79.843
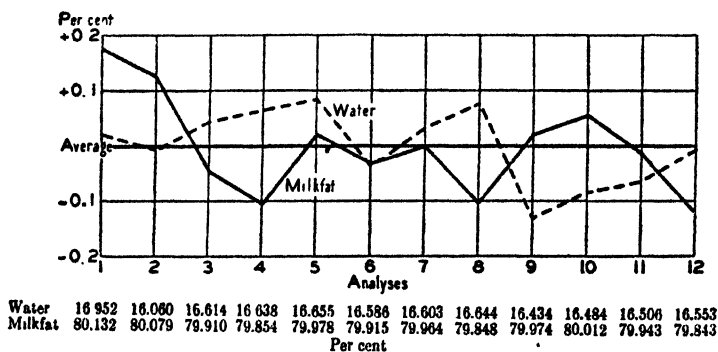
Per cent

GRAPH 2. VARIATIONS WITHIN A SINGLE SAMPLE OF BUTTER

(Twelve analyses by the A. O. A. C. method)

again weighed. The second reading, as may be seen in graph 1, had a mean variation in milkfat of 0.117 ± 0.007 per cent, with a range from 0.001 to 0.337 per cent. The water had an average difference, out of the 66 possible variations, of 0.018 ± 0.001 per cent, with a range from 0.001 to 0.051 per cent.

Inasmuch as the modified Kohman method gave very satisfactory results when the butter was weighed in a closed cup, it seemed advisable to again see what the results would be in a second trial of the A. O. A. C. procedure. The butter was weighed in a covered cup and then analyzed. The data in graph 2 show that the mean variation in milkfat was 0.104 ± 0.006 per cent, with a range from 0.004 to 0.289 per cent. The average difference in the water was 0.081 ± 0.004 per cent, with a range from 0.006 to 0.221 per cent.

The manner in which Guthrie (2) executed the modified Kohman method is outlined under "Operation," page 387. The Association of Official Agricultural Chemists (A. O. A. C.) procedure was as follows:

A sample of butter weighing from 1.5 to 2.5 grams was placed in a flat-bottomed dish having a surface of at least 20 square centimeters. It was then dried in a hot-water oven and weighed hourly during the process until the weight was constant.

When determining the milkfat, the whole sample was washed into a Gooch crucible with petroleum ether as a solvent. The washing was continued until the filtrate was free from milkfat. The Gooch crucible then was placed in the hot-water oven to dry. When hourly weighings showed constant weight, the amount of the milkfat was obtained by deduction as follows: water 14.5027 per cent + casein and salt 3.2104 per cent = 17.7131 per cent; 100 per cent − 17.7131 per cent = 82.2869 per cent, the milkfat content.

It should be noted that these studies were made in a well equipped laboratory with balances accurate to 0.1 mgm., and where it was easily possible to place approximately ten grams of butter in the beaker and to cover it immediately to prevent evaporation of moisture, and then to ascertain the exact weight.

Conditions, however, in the creamery are quite different, for the balances are standardized to weigh exactly 10 grams. It is necessary, therefore, to work carefully and rapidly in making the weighing without much loss of moisture while the butter is on the scales. In the average creamery, where the whole procedure is not as accurately conducted as in a well organized laboratory,

it is probably advisable to use simplified equipment such as the Torsion balance where 10 grams of butter are used and where the readings, in moisture, are obtained directly in percentage.   When
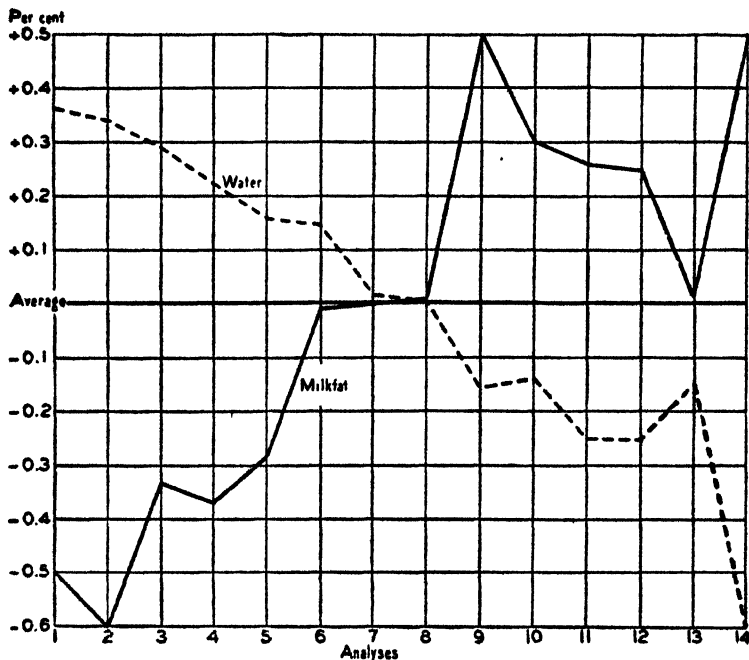
GRAPH 3. VARIATIONS WITHIN A SINGLE SAMPLE OF BUTTER
(Fourteen analyses by the modified Kohman method)

This graph shows the variations that existed between the different analyses in a sample of butter. All of the analyses were made after the butter had been carefully prepared in a 500-cc. wide-mouthed bottle. The graph does not include the variations due to sampling from churn, tub, roll, or print. The weighings were made on a balance that read to 0.1 mgm., which naturally would show less variation than the scales in use in the average creamery. The butter was weighed in an open aluminum cup; this is the practice in most creameries, and is not satisfactory.

running the tests in this manner, the operator of a butter factory should not expect to do closer weighing than is shown in graph 3.

In the studies by Guthrie an electric hot plate was employed. Most creameries, at present, can be supplied with this convenience. But if they do not have electricity nor gas, an alcohol

lamp may be used to evaporate the moisture. When a flame is used, gasoline can not be evaporated over it, because gasoline is very inflammable. Other means of drying in this case are necessary.

When exactly 10 grams of butter are weighed into the beaker, as in case of the usual creamery practice, sufficient moisture evaporates during the weighing process to make a marked error. Graph 3 shows that the variation in water was from approximately −0.6 per cent to about +0.36 per cent. In milkfat the range was from approximately −0.6 per cent to +0.5 per cent. These wide variations were due to the evaporation that took place during weighing. In contrast to this graph, it should be observed in graphs 1 and 2 that the variations are small. The reason for this is that the analyses reported in these two graphs were run under a better laboratory technique, where finer balances were employed, and where approximately 10 grams of butter were placed in the beaker and then weighed under a watch glass. The average creamery operator should expect to do closer weighing than is shown in graph 3, if he uses the equipment listed below, if he puts approximately 10 grams of butter in the cup under a watch glass, and if he is painstaking in his technique.

THE KOHMAN METHOD OF ANALYZING BUTTER

So long as the modified Kohman method of analyzing butter is more simple of operation than the A. O. A. C. procedure, it is being given preference for the operator of a creamery. And in order to particularly assist the creameryman, who is a layman in matters pertaining to chemical analysis, a complete list of equipment and supplies is hereby given, and a discussion of the operation of the test follows.

Equipment and supplies

A large spatula and a small spatula. The larger one may be made of hardwood, about $\frac{1}{16}$ by $\frac{1}{2}$ by 10 inches in dimensions. The smaller one should be of thin metal. A pocket knife will suffice.

A sample jar, in which the butter is thoroughly stirred to a
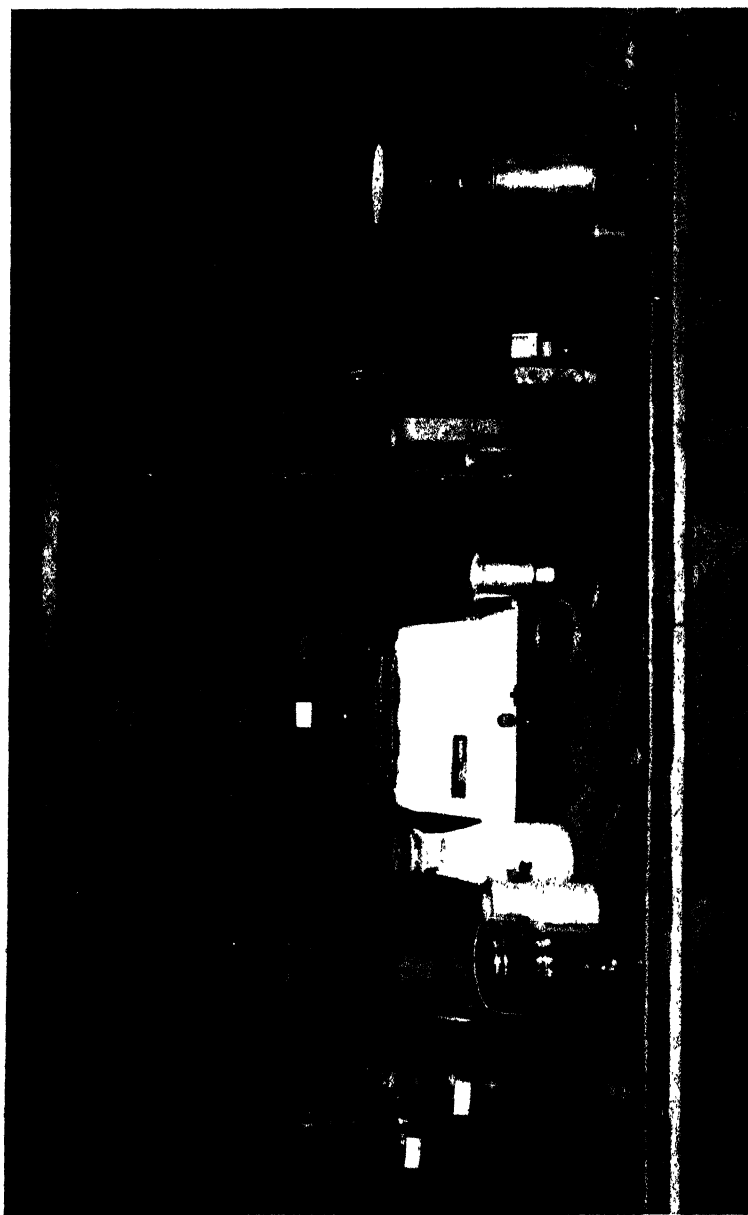
FIG. 1. EQUIPMENT AND SUPPLIES FOR THE MODIFIED KOHMAN METHOD OF ANALYZING BUTTER

creamy consistency, which may be a pint milk bottle fitted with a number 9 rubber stopper. For an alternative method of preparing a butter sample, a pint fruit jar that is fitted with a rubber ring may be employed. In this second procedure (3) the butter is melted and then it is vigorously shaken during the cooling process.

A metal beaker or dish approximately 2 inches in diameter and 3 inches high. A small pair of forceps to handle the weights and to shove the riders back and forth on the beams. A larger pair of forceps, for lifting the beaker, was unintentionally left out of figure 1.

A set of weights weighing from 1 mgm. to 100 grams, that costs about $5. A torsion balance valued at about $40, which may be kept in a home-made cabinet. A tripod with an asbestos mat. An alcohol lamp. A box of matches. A supply of gasoline, such as is found in all automobile filling stations. A thin metal shelf. A cooling surface such as an old flatiron or a smooth building stone, or concrete.

A roll of tissue. A rubber-tipped glass rod or soft-wood stirrer. A supply of silver nitrate. A burette. A bottle of potassium chromate. A pipette. A graduated cylinder or a flask. A white cup and a stirring rod with a color scale is convenient.

Operation

1. Obtain the sample. When sampling from the churn the hardwood spatula is satisfactory. The extraneous moisture may be eliminated by scraping off the surface of the butter in the churn, over a small area, and immediately obtaining a portion of approximately 10 to 15 grams. For every-day churn analysis, a sample should be made up of 10 or 12 portions. This butter should be placed in a jar that has been dried over a steam pipe or in a similar way, for at least several hours. The type of jar will depend on the method employed in preparing the sample. When the butter is thoroughly mixed by stirring, a pint glass milk bottle properly fitted with a rubber stopper is satisfactory, for the neck is long and sloping. In a milk bottle, small portions of

butter can not stick in inaccessible places, as is true of bottles with square shoulders.

When the sample is taken from the package and not from the churn, a portion should be obtained from each tub or printer box. This should give a representative sample of the finished product of a single churning. If the sample is obtained during the packing process a portion of soft butter may be obtained from each package before it is finished off. When the sample is obtained from hard butter, a single trierful taken by boring diagonally from the top to the bottom will suffice. About an inch of butter from each end of the trier, in this case, should be eliminated.

2. Balance the metal beaker or dish, placing the metal disc or a watch glass over the top. The doors of the cabinet, during weighing, should be at right angles with the case, so that the balances may not be affected by drafts of air.

3. The sample of butter should now be prepared for weighing. When the butter is brought to a creamy consistency by stirring, it should be warmed in water at a temperature from 100° to 110°F. until the milkfat has almost reached the melted stage. In case the test does not have to be completed quickly, the butter may be softened at room temperatures. If care has been exercised in warming the sample, so that it is not too soft, it will not be necessary to cool it during the latter part of the preparation. Thus, time may be saved. When a sample of butter is being prepared, it should be tightly closed, with the exception of when it is being stirred. During the weighing the jar should be made tight as quickly as the butter has been taken out.

4. By the employment of the large hardwood spatula, place approximately 10 grams of butter in the beaker or dish. The small metal spatula or pocket knife should be used here, to scrape the butter from the large one into the dish. The butter should be landed on the bottom of the dish, and not smeared over the sides. The toilet tissue is convenient to wipe the butter from the spatula, the knife, and other apparatus.

Time should not be taken to weigh exactly 10 grams of butter into the dish, for there will be too much evaporation of moisture

before the weight is obtained.   The metal disc or watch glass should be placed on the dish as quickly as the butter is delivered into it.   This precaution in weighing makes necessary the use of the block of weights that weigh to 0.01 gram.   In case the butter-maker wishes only a close approximation of the composition of his butter, he can save time by weighing out exactly 10 grams of butter.   Then the percentage moisture can be read directly on the beams of the scale.   Thus, he is saved the time of computing the percentage.   To illustrate the obtaining of the amount of butter in the former case, let us say that the beaker weighed 25.48 grams, and that the beaker and the butter amounted to 35.12 grams.   The difference is the weight of the butter, $35.12 - 25.48 = 9.64$ grams of butter.

5. By the use of the large forceps transfer the dish from the balances to the asbestos pad, in case a flame of gas or alcohol is used to drive off the moisture, or to the hot-plate if electricity is the heating medium.   During the drying process, it is desirable to use an asbestos pad over a flame, for otherwise carbon is likely to deposit on the dish and affect the weight.   The employment of rather low temperatures, such as 275° to 300°F. is essential, for at higher temperatures the butter is likely to spatter out of the dish.   At this time it is wise to shake the samples sufficiently often to completely break up the casein blanket.   When this casein surface is not properly broken, particles of it will float away later, on the gasoline.   This causes an error in the analysis.

6. As quickly as the milkfat has reached a golden-brown color, the dish should be placed, with the forceps, on a smooth and clean iron or stone surface to cool.   The forceps should be employed in preference to the hands, for the grease or moisture of the hands might change the weight of the dish.   Iron or stone, which have an extremely high capacity of heat conduction, will rapidly cool the beaker and its contents.   This cooling will be accomplished in 5 minutes, which is about the same as the time consumed in reducing the temperature over an electric air-fan or by cooling in cold water.   When the last method is followed, there is danger of not removing all the moisture from the dish,

even though it is wiped with a dry cloth. It is usually advisable to place a weight on the dish or beaker when it is cooling, so that its contact with the iron will be intimate. The cooling iron should be free from dust, grease, and the like, in order that the weight of the dish will not be affected.

7. The cooled beaker and its contents weighed 33.63 grams. 35.13 grams (weight of beaker and butter in step 4) − 33.63 grams = 1.49 grams (weight of evaporated moisture). The percentage of moisture is $\dfrac{1.49 \text{ grams} \times 100}{9.64} = 15.45$.

8. Fill the cup to within about $\frac{3}{4}$ inch from the top with gasoline, and thoroughly stir it with either a rubber-tipped glass rod or a softwood spatula. The purpose of the gasoline is to dissolve the milkfat. After it has stood approximately three minutes, the salt and casein will have settled to the bottom. Then the gasoline with its load of milkfat may be carefully poured into the drain. The beaker must be handled gently, so that the salt and casein will remain at the bottom. If little flakes of casein float on the surface of the gasoline, they are an indication that the casein film was not properly broken by agitation during the process of the evaporation of the moisture, as pointed out in step 5.

9. Again fill the beaker with gasoline and thoroughly stir the mixture. After standing another three minutes this gasoline may be poured off. Where it is wise to be economical in the use of gasoline, this second washing may be saved, to be used in the first extraction in the succeeding analysis. At this stage about 2 cc. of gasoline will remain in the beaker.

10. If an electric hot plate is employed, the beaker or dish with the remaining salt and curd may be dried by placing it immediately on the plate. In case the evaporation must be accomplished over a flame of gas or alcohol, the remaining gasoline should be largely dried off over a steam pipe or a radiator, for gasoline is too inflammable to be placed directly over a flame at this stage of the analysis. The thin metal shelf shown in figure 1, may be placed over the steam line for convenience in holding the beaker at this period of the process. The completion of the

drying process may be accomplished over a low flame when an asbestos pad is employed. The contents of the beaker when evaporation is completed, should be largely in the form of a dry powder.

11. Cool the beaker and its contents and then weigh. The calculation of the amount of milkfat is done as follows: 33.63 grams (weight before milkfat was extracted) − 26.19 grams (weight after milkfat was extracted) = 7.84 grams of milkfat.

The percentage of milkfat is $\dfrac{7.84 \text{ grams} \times 100}{9.64 \text{ grams (weight of butter)}}$ = 81.32.

Fig. 2. The Troy Salt Test

12. In preparation for determining the amount of salt, transfer all of the contents of the beaker to the 300 cc. flask, in case of the Troy salt test, or a 250 cc. flask if the Nafis salt test is employed. This transfer may be accomplished by filling the beaker approximately three-fourths full of lukewarm water and stirring the mixture with a rubber-tipped glass rod or a softwood spatula. Then after pouring the contents of the beaker into the flask, the procedure should be repeated until all of the salt and curd have been transferred to the flask. Now the flask should be filled to the 300 cc. or 250 cc. mark with warm water.

13. Shake the flask sufficiently long for the salt to be uniformly

distributed throughout the solution. Now measure out 17.6
cc. of the salt solution when the Troy salt test is being used, or
25 cc. if the Nafis test is being employed; and put it into a white
cup for titration.

14. After putting 2 or 3 drops of indicator (potassium chromate)
into the salt solution in the white cup, titrate into it the silver
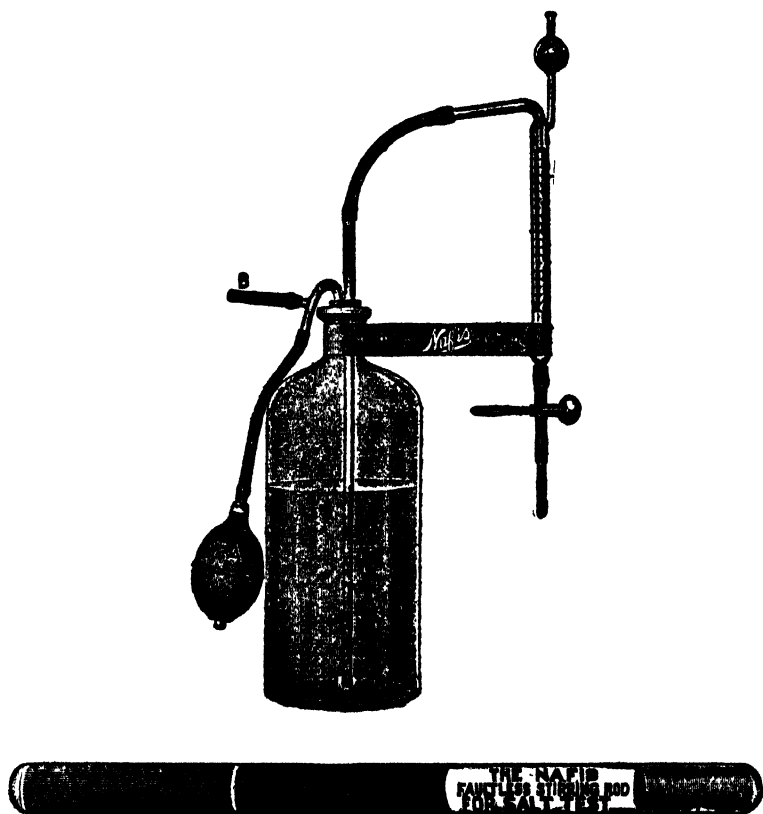
FIG. 3. THE NAFIS SALT TEST

nitrate from the burette to a brownish-red color.   In the Troy
salt test the silver nitrate is a tenth normal solution, and in the
Nafis salt test it is a special strength but in both cases the dilu-
tions are calculated so that the cubic centimeters of silver nitrate

used is equal to the percentage of salt. In this analysis it was 2.5 per cent.

15. The following procedure is followed in calculating the percentage of curd. The sum of the moisture, 15.45 per cent, milk-fat 81.32 per cent, and salt 2.5 per cent, is 99.37 per cent. 100 per cent − 99.37 per cent = 0.63 per cent curd.

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SUGGESTED METHODS FOR THE MICROBIOLOGICAL ANALYSIS OF BUTTER

FOREWORD

The American Dairy Science Association, through its committee on bacteriological methods, is contemplating the formulation of a complete set of microbiological analytical procedures useful in controlling the quality of dairy products. This committee expects to act largely through sub-committees appointed from men in the Association who have had experience with the bacteriological analysis of various dairy products. Obviously, the formulation of such methods should not be left to the arbitrary decision of a committee, but should be the result of suggestions and criticisms coming from all interested parties, whether or not they are members of the American Dairy Science Association. The committee, therefore, wishes to serve as a center about which methods satisfactory to the largest number may be evolved.

The rapid development in the use of laboratory methods in the control of butter manufacture has made it necessary to study the various methods now used in order to adopt uniform procedures (which at the present time do not exist) for the control of microörganisms in butter. These methods will be revised later in accordance with the suggestions received and the judgment of the committee, before they are finally adopted by the American Dairy Science Association and included in the report on bacteriological methods of analyzing dairy products.

THE AGAR PLATE METHOD FOR THE MICROBIOLOGICAL ANALYSIS OF BUTTER

Sterilization of glassware. Same as outlined in the latest edition of Standard Methods of Milk Analysis, American Public Health Association, 370 Seventh Avenue, New York City.

Sterilization of equipment used in sampling. Metal triers, spatulas, spoons to be wrapped in paper (Imported Kraft wrap-

ping paper usually withstands sterilization temperatures without charring), or enclosed in metal containers and sterilized in the same manner as glassware, or in the autoclave at 15 pounds steam pressure for 30 minutes.

Wooden tongue depressors serve as satisfactorily as spatulas. They may be wrapped in paper or enclosed in a metal container and sterilized by autoclaving, or by hot air.

Method of sampling. 1. Butter in the churn. After the butter has been worked and is ready to be removed from the churn, samples for microbiological analysis should be taken by means of a sterile trier, taking three samples of about 1 ounce each, one sample from the center of the churn, the other two from the respective ends.[1] The butter should then be placed in sterile screw top sample jars.

2. Tubs or packages. About 1 ounce of butter should be removed from two different parts of the tub or package with a sterile trier, the plugs of butter to be not less than 2 inches in length and to include the surface portion. The butter should be transferred from the trier to the sterile screw top sample jar with the aid of a sterile spatula or spoon.

3. Print butter. Because of difficulty in obtaining the same amount of exposed surface of butter on one, one-half and quarter pound prints, the method of sampling print butter should be by means of a trier. By using a small trier and plugging the end of the print, a plug of 3 to 4 inches may be obtained. Such a plug will weigh about half an ounce. The butter should be transferred from the trier to a sterile screw top sample jar with the aid of a sterile spatula or spoon.

Care of samples. All samples should be placed in cracked ice immediately after sampling or placed in a refrigerator where the temperature does not exceed 4°C., and plated as soon as possible.

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[1] Under commercial conditions where large numbers of samples must be taken daily, it may be impractical to employ a sterile trier for each sample. If a polished trier is wiped thoroughly after each sample with tissue paper until the surface is highly polished and then plunged into the butter to be sampled at least twice before the sample is taken, satisfactory results can be obtained with a single trier.

Where samples are procured from a grading station, remote from the laboratory, the sample should be stored at −10°C., or lower until shipped.

For shipping, the samples should be placed in a precooled iceless ice cream shipping container and packed carefully so as to prevent breakage. Upon receipt of the samples at the laboratory they should be plated as quickly as possible and record should be made as to the number of days elapsed between sampling and plating.

Preparation of samples for plating. Place the sample jar containing the butter to be tested in a water bath between 40 and 45°C., and shake until the butter is of a creamy consistency. The time required for the sample to reach a creamy consistency should not exceed 15 minutes. Plating should follow immediately.

Preparation of dilutions. With a previously warmed 1 cc. pipette,[2] the melted butter should be drawn up into the pipette, care being taken to have as little butter as possible on the outside of the pipette. To remove as much butter as possible from the pipette, blow hard through the pipette into the dilution blank which has been previously warmed to a temperature of 40 to 45°C. Rinse the pipette by sucking the dilution water into the pipette.

DETERMINATION OF YEASTS AND MOLDS

Media:

1. Bacto Dehydrated Whey Agar.
2. Bacto Dehydrated Malt Agar.
3. Whey Agar made according to one of the following formulae:
    I. Coagulation with Acid.
        1. Use fresh, skimmed milk.
        2. Place skimmed milk in water bath and hold at a temperature of 35°C. while adding dilute hydrochloric acid to precipitate casein. (To prepare the dilute acid, add 1 part of hydrochloric acid containing 31.45 per cent hydrochloric acid, sp. gr. of 1.16 or 20 degrees Baumé, to 8 parts

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[2] The specifications for the pipette to correspond with the specifications of milk pipettes as given in Standard Methods of Milk Analysis.

of distilled water. To prepare 1.16 hydrochloric acid from stock concentrated acid, dilute 29.3 cc. with 5.2 cc. of distilled water. To prepare the acid for precipitating, dilute the resulting mixture with 276 cc. of distilled water.)

3. Bring whey to a pH of 4.6 (Colorimetric or potentiometric method may be used.)
4. Filter whey through cheese cloth to remove curd.
5. Place whey in autoclave for 15 minutes at 15 pounds pressure.
6. Filter through absorbent cotton.
7. Neutralize filtrate with N/1 NaOH to a pH of 6.8 to 7.0.
8. Add 0.5 per cent peptone and then autoclave for 15 minutes at 15 pounds.
9. Filter through filter paper.
10. Add 1.2–1.5 per cent agar to whey broth and heat in steam bath to melt agar.
11. Filter through cotton if necessary.

II. Coagulation with Rennet.

1. Use fresh skimmed milk.
2. Heat to 38° to 40°C. Add about 2 cc. rennet extract per liter and allow the milk to curdle firmly. Break up the curd thoroughly so that it will contract rapidly, heat to about 55°C. to aid this contraction, and separate the whey by straining through cheese cloth.
3. Weigh out peptone equal to 0.5 per cent of the whey and agar equal to 1.5 per cent of the whey. Add these to one-half the whey, weigh and then heat until the added ingredients are dissolved and restore the water lost. Combine the two portions of whey and adjust the pH to 6.4 to 6.8 and the temperature to about 55°C.
4. Add egg (in the proportion of 1 whole egg to 2 or 3 liters of medium) and distribute thoroughly by stirring and then pouring the medium from one container to another.
5. Flask and autoclave at 15 pounds pressure; the time of heating is dependent on the size of the flasks.
6. The medium may be filtered through cotton and tubed or bottled at once, or it may be stocked in the flasks and melted, filtered, and tubed, or bottled as needed.

The media should be sterilized in definite quantities, preferably 100 cc. by autoclaving at 15 pounds steam pressure for 20 minutes.

Acidulating the medium. Adjust the reaction of the warm sterile agar to a pH of 3.5 with sterile lactic or tartaric acid solution before pouring. Never heat the agar after the addition of the acid, as heating at an acidity of pH 3.5 may destroy the jellying properties of the agar.

The amount of lactic or tartaric acid to be used varies according to the buffering qualities of the medium. Five per cent acid solution is recommended.

Colorimetric or potentiometric methods should be used to determine the amount of acid necessary to bring the pH of the medium to pH 3.5.

By using the indicator brom phenol blue, a yellow green color is obtained at pH 3.5.

As an index as to the amount of 5 per cent lactic acid of 85 per cent purity required, Hood has compiled the following table:

MEDIUM	APPROXIMATE AMOUNTS OF 5 PER CENT LACTIC ACID SOLUTION TO 100 CC. OF MEDIA	FINAL pH
	cc.	
Bacto Dehydrated Whey Agar..........................	5.5	3.5
Bacto Dehydrated Malt Agar........................	2.5	3.5
Whey Agar (homemade)................................	10.0	3.5

Table taken from Studies on Moulds and Yeasts in Creamery Butter Pamphlet 92, New Series, Department of Agriculture, Dominion of Canada, 1928.

Dilutions. Dilutions suggested for determining the yeast and mold content of butter are 1, 0.1 and 0.01 of a cc. Higher dilutions may be made if desired.

Plating. About ten cc. of acidulated melted agar cooled to a temperature of 40 to 45°C. should be poured into each petri dish after the introduction of the sample and thoroughly mixed with the sample by a rotary motion.

The plating should be completed as rapidly as possible.

After the medium has thoroughly hardened, invert the petri dishes and place in incubator.

Incubation. The plates should be incubated at either 21° or 25°C. for 5 days.

The plates should be examined after two or three days incubation to determine the amount of mold growth and, if the molds are developing in large numbers, a count should be made at this time and a recount on the fifth day.

Counting. Count the number of mold and yeast colonies without the aid of a counting lens.

Reporting results. The number of yeast and mold colonies per cubic centimeter should be reported separately, and together, as a total count. The kind of medium used should be reported. Where media are clarified with egg this fact should be stated.

TOTAL BACTERIAL COUNT

Medium. The following media are suggested for the determination of the bacterial count in butter:

1. Bacto Peptonized Milk Agar.
2. Whey Agar—pH 6.6 to 6.8.
3. Beef Infusion Agar, containing 1 per cent lactose.

For the preparation of Beef Infusion, see General and Pathogenic Bacteriology, Moore and Hagen, P. 9, Ginn & Company, New York, 1925. The above media should be adjusted to a pH of 6.8.

Dilutions and plating. According to Standard Methods of Milk Analysis, using dilution blanks at a temperature of 40° to 45°C.

Incubation. (*a*). Five days at 21°C. or (*b*) four days at 21°C. and 1 day at 37°C.

Counting. According to Standard Methods of Milk Analysis.

Expression of results. The results as to the total number of colonies per cubic centimeter of butter should be expressed as total count. The kind of medium used and the temperature of incubation should be reported.

PROTEIN DIGESTING BACTERIA

There are two methods suggested for the determination of protein digesting bacteria in butter. They are:

1. By picking colonies from the plates that have been used to determine the total number of bacteria in butter and inoculating

these colonies into tubes of sterile milk containing either brom-cresol-purple or litmus.

For the preparation of the indicator brom-cresol-purple, see Journal of Agricultural Research, Vol. 10, No. 3, p. 103–111, July, 1917.

All colonies should be picked from the plate or contiguous colonies from a given sector of the plate.

Incubation of tubes should be for 5 days at 21°C.

Expression of results. Results are to be expressed as the proportion of colonies picked that show proteolysis in milk.

2. By using a medium that contains casein and noting the proteolysis as it occurs on the plate.

The following media are suggested:

A. Milk agar:

		grams
a. Agar ...		15 0
Water		900.0
b. Skimmilk		100 0

Flask separately in portions for plating; sterilize, cool to 45°C. and pour plates. The medium should have a pH of 6.6–6.8.

After incubation, flood the plate with dilute acid (1 part concentrated hydrochloric acid to 10 parts of water). If clearing remains, it denotes true hydrolysis of the casein, if not, it is a weak acid clearing.

B. Casein agar, (Frazier, Wm. C. and Rupp, P.: Studies on the proteolytic bacteria of milk. Journal of Bacteriology, Vol. 16, 57–78, 1928).

Incubation. Five days at 21°C.

Expression of results. The number of proteolytic colonies per cubic centimeter of butter should be reported with a record of the medium used.

AN ADAPTATION OF THE FROST LITTLE PLATE METHOD FOR THE RAPID QUANTITATIVE DETERMINATION OF MOLDS AND YEASTS IN CREAMY BUTTER

Mr. C. K. Johns, of the Division of Bacteriology, Dominion Department of Agriculture, Ottawa, has worked out the follow-

ing modification of the Frost Little Plate method for determining the mold and yeast count of butter:

Reference: A rapid method for determining the mould and yeast count of butter. Sci. Agric., 8, 353–369, 1928.

Sampling. Same as outlined for the agar plate method.

Preparation of samples. Same as outlined for the agar plate method.

Care of sample. Same as outlined for the agar plate method.

Preparation of dilutions. Ten cubic centimeter portion withdrawn with a sterile warmed pipette, taking care to remove the butter from the outside of the pipette as completely as possible. Contents then introduced into dilution bottle containing 40 cc. water at 45°C. Rinse the pipette by sucking up dilution water.

Preparation of slide. An area 2 by 4 cm. is marked upon a clean microscopic slide, using a wax pencil and guide. Slide is then placed on a warm table maintained at 45°C.

Medium. Same as outlined for yeasts and molds in the agar plate method. When ready to prepare micro-plate, place 4 drops of medium upon slide.

Making micro-plate. After thorough agitation of the dilution a portion is quickly withdrawn with a sterile 1 cc. pipette graduated in tenths. Two-tenths of a cubic centimeter is placed upon the slide, mixed with the medium by means of a sterile needle, and spread uniformly over the measured area of the slide. The warm table prevents the agar from solidifying before the operation is completed. The micro-plate is then removed to a cold level surface and protected from air contamination while hardening.

Incubation. The hardened micro-plates are placed in a moist chamber, which contains sufficient moisture to prevent the drying out of the agar during incubation. The moist chamber containing the micro-plates is then removed to the incubator. Incubation at 25°C. for 15 hours gives the best results. If a shorter period must be used, the temperature may be raised a few degrees; if a longer period is more convenient, the temperature may be slightly lowered.

Staining. At the conclusion of the incubation period the microplates are removed and dried on a metal sheet placed over boiling

water. This operation should take about 5 to 10 minutes; if dried too fast or left drying too long the film has a tendency to crack. When dry the micro-plates are placed in Coplin jars containing thionin stain made up as follows:

Thionin[2]..	1	gram
Carbolic acid...	2.5	grams
Distilled water... ..400		cc.

Mix, filter and add 5 per cent glacial acetic acid.

Slides should be stained for 3 minutes, then carefully washed in clean water and dried.

Adjustment of microscope. Microscope should be adjusted so that the area of the field under the low power objective will be approximately 2 sq. mm. For details regarding calibration of microscope see latest Edition of Standard Methods of Milk Analysis of American Public Health Association.

Counting colonies. After a preliminary survey to observe the evenness of distribution of the colonies, 20 representative fields are counted. If no colonies are encountered in 20 fields, another 20 may be counted. If the mold and yeast count runs over 50 colonies per field the counting of 10 fields is sufficient. Yeast and mold counts are made in duplicate and averaged. The count per cubic centimeter is then calculated according to the following formula:

$$\frac{A}{B} \times X \times Y \times Z = N$$

where A = number of colonies observed, B = number of fields counted, X = reciprocal of dilution, Y = reciprocal of quantity plated, Z = microscopic factor, N = number of colonies of molds (or yeasts) per cubic centimeter. *E.g.*, if the microscopic factor (area of micro-plate area of field) is 400, and the total count on 20 fields gives 4 molds and 30 yeast colonies we would have

$$\frac{4}{20} \times 5 \times 5 \times 400 = 2{,}000 \text{ mold colonies per c.c.}$$
$$\frac{30}{20} \times 5 \times 5 \times 400 = 15{,}000 \text{ yeast colonies per c.c.}$$

Where the microscopic field is adjusted to 2 square millimeters and 0.2 centimeter of a 1/5 dilution plated, as in the example

[2] Thionin, synonym Lauth's Violet, not Thionin Blue.

care not to produce an emulsion. Too vigorous or prolonged shaking may produce an emulsion which is hard to break.

5. The mixture is allowed to stand until the fat and ether separate and rise, leaving the milk below. The milk will contain the microörganisms.

6. The milk is drawn off into a sterile test tube or flask, and if not clear, is again treated with a fresh portion of ether. It may be necessary to repeat this operation several times.

7. A measured amount of the milk is spread over a definite area upon a microscopic slide, dried, fixed, cleared of fat, if necessary, with xylol, and stained with a suitable stain, such as carbol-erythrosin or carbol-thionin.

8. The preparation is examined under the oil immersion lens, the number of bacteria per field counted, and the number per cubic centimeter of milk, and per gram of butter calculated.

The factor for calculating the number of bacteria per gram of butter from the number per microscopic field is obtained as follows:

Multiply the number of bacteria per field by the number of fields per square centimeter, and multiply this product by the number of square centimeters over which the milk is spread. This gives the number of bacteria in the amount of milk used. Divide this by the amount of milk used, to obtain the number per cubic centimeter of milk, and divide by the number of grams of butter represented by one cubic centimeter of milk.

Remarks. This method is essentially the same as that used for the direct microscopic examination of milk. The method is open to the same objections and limitations as the method for milk, with the added difficulty of the removal of the fat. It has the advantage of rapidity over the cultural methods.

care to avoid foam formation. The fat solvent should also be added a little at a time to prevent resolidification of the fat.

5. After dissolving the fat, the contents of the funnel are allowed to separate and the aqueous portion drawn off into a thoroughly clean beaker.

6. One fiftieth (1/50) of a cubic centimeter (after thorough mixing) is spread out with a needle on a clean glass slide so that it covers an area of one square centimeter.

7. The smears are now treated in the same manner as milk smears for direct microscopic counting. The procedure is outlined in the latest edition of Standard Methods of Milk Analysis.

One-fiftieth of a cubic centimeter of the aqueous portion represents approximately one hundredth of a gram of butter.

A MICROSCOPIC METHOD FOR THE DETERMINATION OF YEASTS AND OIDIA IN CREAM AND BUTTER

Reference: H. W. Redfield. The determination of yeasts and oidia in cream and butter. Jour. of Dairy Science, 5, 14–21, 1922.

By means of a small butter trier, 3 or 4 cores from different portions of the butter sample are transferred to a 6 by 1 inch test tube, usually about half filling the tube. The butter is melted at 45°C. and allowed to stand at temperatures between 45° and 40°C. until the fat is separated in a clean layer with the mixture of curd and whey or brine at the bottom of the tube. With a sterile 1 cc. pipette the curd and whey are thoroughly mixed by drawing the liquid back and forth in the pipette. Then 1 cc. of this mixture is transferred to a clean watch glass care being taken to wipe thoroughly all the fat from the outside of the pipette with a clean towel before discharging the pipette into the watch glass. A Petri dish may be used but is not as satisfactory. With a Breed pipette 0.01 cc. of the mixture is transferred to a microscopic slide, carefully spread over an area of 1 sq. cm., 2 sq. cm., or 4 sq. cm., depending on the quality of the butter, diluting if necessary with distilled water in order to obtain uniform smears. After drying in the air, extracting with xylol, and fixing with 95 per cent alcohol and the slide is stained and dried in the air.

Counting is done with a combination of lens and draw tube length to give a factor of 500,000. The diameter of the field should be 0.16 mm. to give a factor of 500,000. In enumerating yeasts and oidia each cell, whether separate or as an element in a chain or budding colony, is counted. The number of fields to be counted depends upon the number of organisms present. It is recommended that 100 fields be counted except in very high count products.

CONCLUSION

The views expressed in this report are those of a committee appointed by the American Dairy Science Association. As such they are printed for the general criticism of other members of the A. D. S. A., and other interested parties.

Separate copies of this report, and of the Ice Cream Report previously issued may be secured at cost from the chairman of the Committee on Bacteriological Methods.

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THE EFFECT OF INITIAL COOLING TEMPERATURE ON GELATIN IN THE AGING OF THE ICE CREAM MIX*

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Gelatin has generally been used in ice cream to improve the texture by partially preventing the formation of ice crystals. The partial occlusion of water by the gelatin also results in a product which is less watery in consistency. However, the swelling property of gelatin and its behavior in ice cream are not thoroughly understood. Obviously, therefore, if more were known about gelatin, its effect on ice cream could be more readily explained.

The fact is generally known that gelatin will swell to many times its volume in water by physical combination, although the extent of true hydration of gelatin as shown by Theones (1), Adair (2), and Moran (3) is less commonly known. Except for Theones's results they determined that each gram of gelatin combines with less than its own weight of water or 1.86, 0.6, and 0.53 gram of water respectively. Even the latter figure is considered equivalent to 1500 molecules of water per molecule of gelatin, according to Moran.

The above figures for physical and chemical combination appear very high and therefore seem to preclude further study of such constants until those factors are investigated which often cause unexplainable behavior in gelatin solutions. This is necessarily so when one considers that notable increases in the viscosity of an aged ice cream mix are not always proportional to the small quantity of gelatin used. Thus an understanding of gel structure can result only following a consideration of the factors dealing with the formation of a new phase as related to a protein colloid.

The process of aging has long been regarded as a colloidal

phenomenon. Although there is no reference in the literature which satisfactorily explains the process of aging, the results of the process are quite apparent. Aging is normally accomplished by rapidly cooling the mix to about 40°F. after homogenization. By holding at this temperature for twenty-four hours, an increase in viscosity is obtained which approaches the maximum obtainable under the condition stated. Although the degree of viscosity thus attained has been looked upon somewhat as a measure of the completeness of aging, no direct relation has been established between this viscosity and the improved whipping property of the mix and the smoother texture of the finished product. These latter effects are, however, variable to the extent of insignificance.

Further evidence of the lack of practical knowledge regarding aging may be noted by comparing the following methods of treating the mix. Turnbow and Raffetto (4) state that the mix aged at 32°F., not to exceed 36°F., is of better quality and has more viscosity than one aged at higher temperatures. The other extreme is evident in a study by Lucas and Scott (5) in which they state that a hot gelatin solution was added when the mix had cooled to 70°F. Such a method is different in that complete sudden cooling is not possible. From the above it is quite apparent that a better understanding of aging is needed.

Only mixes containing gelatin are appreciably influenced by aging. Thus, a better conception of the factors bearing on the aging phenomenon should be obtained with the aid of a good theory of gelatin structure. Until comparatively recently the structure of gelatin was regarded as a fibrous network by Bogue (6) and others. However, Ostwald (7) summarized theories on "gel" formation and elucidated the theory that "gel" structure is a secondary fusion of enlarged particles. The same idea is substantiated in a recent study by Krishnamurti (8), a student of Donnan, on the scattering of light in colloidal sols and gels. In this study the intensity depends on distinct changes in the shape of the particles. There was no rapid change observed either during gel formation or as a result of agitation of a gel. This indicates that the particles were spherical, because any other shape would show variations when the axis of the particle is moved with

respect to a plane of light. This is especially marked in the case of a rod shape particle. Furthermore, these facts substantiate the work of Bradford (9) who obtained microphotographs of gelatin crystals which he calls spherites. The above conception would indicate, therefore, that the real or basic viscosity of ice cream mix is due largely to the number and size of the particles.

In the aging of ice cream certain properties affecting the size of gelatin particles have been overlooked which, theoretically, have a bearing on the mix. Gelatin is influenced by the phenomenon of hysteresis which is characterized by a difference between the solidifying point and the melting point. These points are affected

TABLE 1

The effect of basic viscosity on the overrun of the ice cream mix, as due to the influence of the initial aging temperature on gelatin content

	BATCH 1	BATCH 2	BATCH 3	BATCH 4	BATCH 5
Initial aging temperature, degrees F....	46	70	81	100	107
Viscosity, degrees M....................	53	184	190	210	185
Mix temperature at the tenth minute, degrees F.............................	24.5	24.7	24.6	24.4	24.4
Overrun at tenth minute, per cent.......	99	95	92	90	90

by the rate of cooling, the significance of which has been brought out by Arisz (10). He observed that a gelatin solution at 68°F. solidified much sooner than a similar solution at 35°F. Arisz interprets his finding as a condition in which the equilibrium necessary to a change of state could take place at the higher temperature more readily than at the low temperature. This is so because the heat influences the molecular activity necessary to the formation of nuclei and particles which in turn determine the basic viscosity due to the size and number of particles.

Theoretically, the observation is extremely significant with respect to aging the ice cream mix inasmuch as the mix is supercooled with respect to the gelatin content. Hence, the development of viscosity with aging is the result of the tendency of the gelatin to attain equilibrium essential to a change of state. The purpose of this study will therefore be an attempt to determine the significance of the initial cooling of the mix and its relation to aging, its effect on viscosity, and on the texture of ice cream.

The mixes, varying in fat from 10 to 15 per cent and in serum solids from 9 to 10 per cent, contained 15 per cent sugar and 0.35 per cent gelatin (250 Bloom test) which was dispersed in cold skimmilk and cream. The sugar and skimmilk powder were then mixed and added. All mixes were pasteurized at 149° to 151°F. for thirty minutes. Homogenization followed at a pressure of 2500 pounds. The mixes were rapidly cooled over a surface cooler to the temperatures subsequently indicated and then were allowed to cool without agitation for twenty-four hours in a 40° cold room.

Preliminary viscosity trials with 200 cc. quantities indicated that the viscosity results with ice cream mix were far more comparable if the plastic structure as distinguished from basic viscosity by Leighton and Williams (11) was broken down by agitation with a high speed stirrer at about 65°F. until the viscosity readings were constant when observed at 68°F. Two minutes was found to be a sufficient period of agitation. DePew (12) describes a similar method for destroying the apparent viscosity. Disregard for apparent viscosity nullifies precise methods of calibrating a viscosimeter.

The viscosity was determined after twenty-four hours with a MacMichael viscosimeter using a No. 30 wire with the disc bob. The cup was rotated 19.1 r.p.m. Readings were taken when the temperature in the cup reached 68°F. All readings are stated in degrees M. After aging, the mixes were frozen in a forty-quart brine freezer when freezing data were required. A power driven hand freezer was satisfactory when accurate freezing data were not required. The fat and total solids were determined by the Mojonnier method.

The general effect of initial aging temperature on the viscosity of a solution containing gelatin is shown in figure 1. The data are plotted for viscosity obtained by allowing 200 cc. quantities of skimmilk containing 0.5 per cent gelatin to cool slowly from the

temperatures indicated to 40°F. Likewise every mix showed similar increases in viscosity depending on the initial aging temperature except that mixes without gelatin did not develop extra viscosity when initially aged at a high temperature.

Table 1 is a record of data obtained by slowly cooling 45-pound batches of mix without agitation from the temperatures indicated to 40°F. The table also shows the effect of viscosity on the percentage volume increase or overrun attained in 10 minutes. The figures show that the mix temperatures are practically constant.

The photograph, figure 2, shows the appearance of the melting samples at various intervals of time in a series of samples frozen
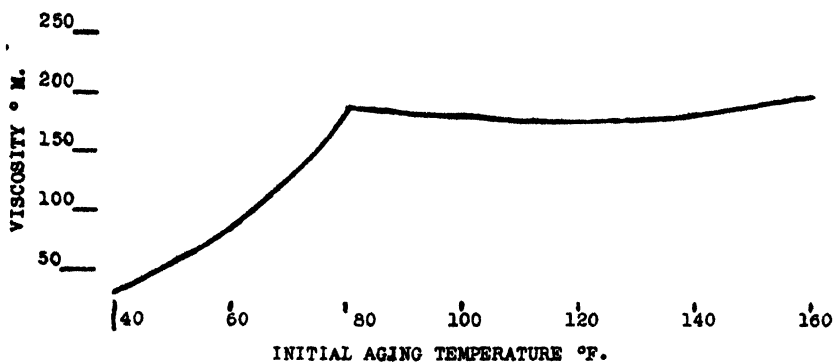
FIG. 1. EFFECT OF INITIAL AGING TEMPERATURES ON VISCOSITY OF 0.5 PER CENT GELATIN IN SKIMMILK

from batches initially aged at the temperatures indicated. While numbers 1, 2, and 3 show very definite progression in the rapidity of melting, numbers 4, 5, and 6 appear to be more constant in character of melting which tends to conform with the corresponding viscosity results. The melting samples of all temperature series studied showed the same general characteristics both in rate and manner of melt which progressed from smooth to rough in the direction of the high temperature.

The results in the foregoing figures and table are quite typical for a large number of mixes with respect to the trend of the curve. All the curves are characterized by an abrupt rise to approxi-
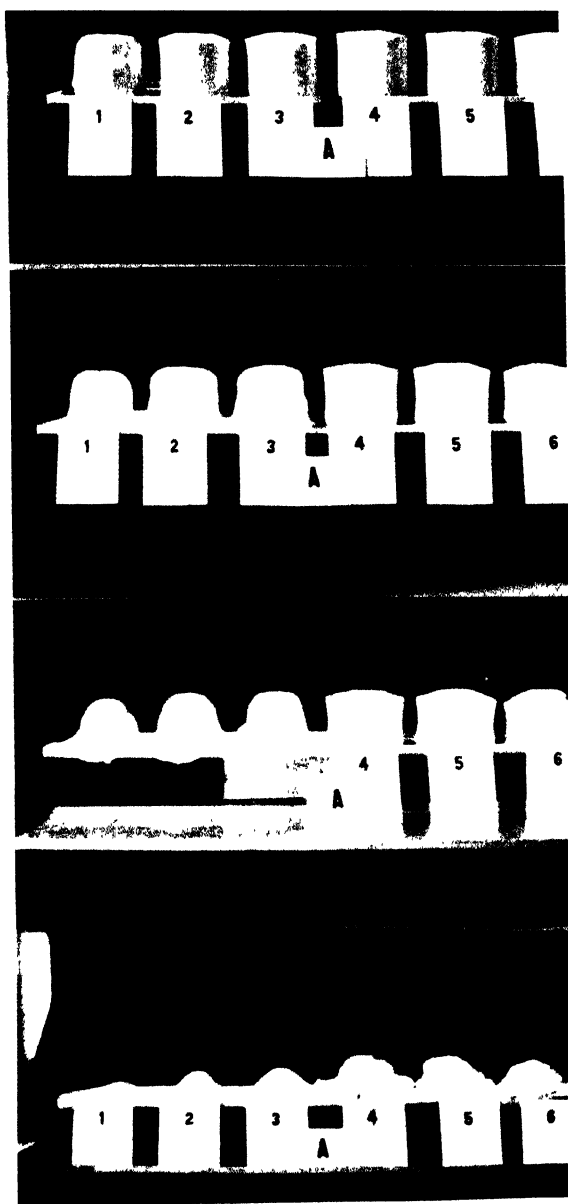
FIG. 2. THE APPEARANCE OF MELTING SAMPLES AT VARIOUS INTERVALS OF TIME

Sample number ..					1	2	3	4	5	6
Initial aging temperature....... ..	.	40	50	60	85	95	105
Viscosity, degrees M	...	.....	100	120	130	260	230	231

mately 80°F. This rise varied from 50 to 400 per cent greater than the 40°F. viscosity result. Many of the series developed maximum viscosity at 80°F. while a few increased slightly in the case of the 100°F. initially cooled mix. Above this initial temperature, all mixes showed a decrease in viscosity with the exception of a few within the limit of experimental error. The rise in viscosity was accompanied by improvement in texture in the finished product.

The initial cooling temperature was not noted beyond the first observation because the agitation required to obtain a uniform temperature of a given portion would hasten cooling and possibly disturb gelation.

A more logical procedure is now being followed in the continuation of this study. Portions of a mix are held at the various temperatures for specific periods of time and are then rapidly cooled to 40°F. For example, maximum viscosity was developed in less than three hours by holding at 80°F. This method makes it possible to obtain a more definite idea of temperature effects as well as temperatures less favorable to bacteria.

In regard to bacteria, their growth and acidity development were considered to be very minor factors in comparison with the fundamental kinetic activity concerned in this study. Hence, bacterial counts and acidity data were not closely observed. The above viewpoint is taken from several different angles, including the fact that in at least one instance over 100 per cent increase in viscosity was noted between samples initially aged at 35° and 50°F. respectively.

DISCUSSION OF RESULTS

The characteristic rise in viscosity accompanying conditions of higher initial aging temperatures appears to be in perfect agreement with the principles of Arisz; namely, a condition permitting equilibrium of energy necessary in a change of state from a liquid to solid. This change and its relation to viscosity may be more readily understood with a consideration of Freundlich's (13) discussion on "The Kinetics of the Formation of a New Phase."

By interpreting the results obtained in the light of the above, the fact appears that the low viscosity of a mix is the result of a rapid supercooled liquid phase whose molecular activity is so low as to be unfavorable to nucleus formation and particle growth. Thus, the development of molecules to particles is relatively slight and therefore very little viscosity results. Conversely, the high viscosity resulting from a slow cooled mix initially held at 80°F. appears to be the effect of an optimum condition for the production of nuclei and their growth because this temperature favors molecular activity which permits equilibrium of kinetic energy.

If one may here introduce the hypothesis of Von Weimarn (14) that gelation of gelatin is an extreme case of crystallization, the above temperature (80°F) may be called the labile range in terms of crystallography. The range from 80° to 100°F. would be considered the meta-stable area because fewer crystals were formed as reflected by a lower viscosity in most instances. The figure 100°F., the melting (transition) point of gelatin according to Oakes and Davis (15), gives this point on the curves studied theoretical significance because it is linked with a distinct drop in viscosity in most instances. Obviously, a study in which the mix is held at the various temperatures and then rapidly cooled to the final temperature is necessary before accurate facts can be determined regarding the exact relation of crystallization of gelatin in ice cream to the rate of equilibrium.

Although the foregoing is of fundamental interest in explaining to a considerable degree the phenomenon of aging and variations in viscosity observed in the plant and laboratory, the seeker of the purely practical phase may note that considerable viscosity may be developed without seriously affecting the whipping property of the mix or the appearance of the melting frozen product. As to the question of bacterial growth, preliminary trials show that a significant rise in count does not begin until an initial aging temperature above 70°F. is employed. This is under condition of slow cooling and is apparently much more favorable to growth than a short holding period followed by a rapid cooling to 40°F.

CONCLUSIONS

1. The experimental work indicates that a greater part of the texture benefits resulting from aging an ice cream mix are associated with the viscosity imparted by gelatin.

2. A high initial temperature in the aging period favored the development of greater basic viscosity.

3. The maximum viscosity was found to be imparted when the mix was allowed to cool from 80–100°F. to 40°F. without agitation.

4. The whipping property was decreased as the viscosity increased.

5. The rate of melting was decreased as the viscosity increased. The manner of melt-down was also influenced by difference in viscosity.

6. This study indicates that the results of aging an ice cream mix may be dependent upon the factors affecting the crystallization of the gelatin portion of the product.

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RELATION BETWEEN TITRATABLE ACIDITY AND HYDROGEN ION CONCENTRATION OF ICE CREAM MIXES*

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It has long been known that the titratable acidity of milk freshly drawn from the cow does not indicate the true acidity, and that the acidity as it then appears, when determined by means of the ordinary acid tests, is merely apparent acidity. Van Slyke and Bosworth (1) showed that this acidity is due to the presence of substances other than lactic acid in the milk. McInerney (2) noted that milk freshly drawn from the cow gave an acid reaction to titration with an alkali which he calls apparent acidity due to carbon dioxide, acid phosphates and casein. The apparent acidity was found to range between 0.10 and 0.22 per cent and milks high in milk solids-not-fat were found to be higher in apparent acidity than milks of lower milk solids-not-fat content. Rice and Markely (3) found the acidity of cow's milk, calculated as lactic acid, to vary from 0.086 to 0.229 per cent and the hydrogen ion content to vary from pH 6.3 to 7.2, with an average of pH 6.5.

Sharp and McInerney (4) obtained samples of milk which ranged in acidity from 0.05 to 0.50 per cent, calculated as lactic acid and in hydrogen ion concentration from pH 6.0 to 7.73. By plotting the titratable acidity of the samples against the pH values, they found that a definite relation existed, but that the relationship for fresh milk was different from that after lactic acid had formed in the milk due to the action of bacteria.

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The relation of the titratable acidity to the hydrogen ion concentration of ice cream mixes has been given little attention by investigators. Workers have studied one or the other but have not attempted to show their relation. Turnbow (5) studied the hydrogen ion concentration of 100 mixes and found it to vary between pH 6.2 and 6.35.

Knowledge of any relationship between titratable acidity and hydrogen ion concentration would be welcomed by ice cream production men. Many of these men make a practice of standardizing the acidity of any mixes that exceed a certain standard, as determined by the ordinary acid test. The acid content is reduced to a definite acidity, the point to which it is reduced being established more or less arbitrarily by the plant operators. It is possible to find mixes which are quite high in titratable acidity, but which are low in actual acidity. In a case like this any attempts to standardize the acidity by usual plant methods might result in producing an alkaline mix.

Procedure

The mixes studied in this experiment were made for the most part from cream, condensed skimmilk, skimmilk, sugar, and gelatin. Mixes of several distinct compositions were used, but all were normal commercial mixes in every respect. All mixes were pasteurized at 145°F. for thirty minutes and homogenized at a pressure of 2500 pounds at the pasteurizing temperature, cooled to 40°F., aged for twenty-four hours and the determinations made.

The titratable acidity calculated as lactic acid was determined on 9 grams of mix, diluted with 9 grams of distilled water. This diluted mix was titrated with N/10 sodium hydroxide, using phenolphthalein as an indicator. The hydrogen ion concentration was determined electrometrically at 25°C. The quinhydrone electrode was used. Lester (6) determined the hydrogen ion concentration of 10 samples of whole milk, cream and whey with the quinhydrone electrode and also the bubbling hydrogen elec-

trode.   The pH values of the two methods varied, on the average, less than 0.05 pH.

The acidity and pH of normal mixes

The data presented in figures 1, 2 and 3 were obtained from normal ice cream mixes made according to the method just described.   These mixes contained 12 per cent fat but the serum solid content varied from 10 per cent to 12 per cent.   The data are assembled according to serum solid content.   Mixes listed

FIG. 1. TITRATABLE ACIDITY AND pH OF MIXES TESTING 10 PER CENT SERUM
SOLIDS

under 10 and 12 per cent serum solids were made from cream, condensed skimmilk and skimmilk, while some of those listed under 11.7 per cent serum solids contained some unsalted butter and dry skimmilk of various types.   The majority of hydrogen ion determinations fell in the range pH 6.25 to 6.35, with an average fairly close to pH 6.3.

The titratable acidity was highest in the mixes containing the largest percentage of serum solids.   This has been demonstrated before (7) and this knowledge is of importance to the ice cream

FIG. 2. TITRATABLE ACIDITY AND pH OF MIXES TESTING 11.7 PER CENT SERUM
SOLIDS
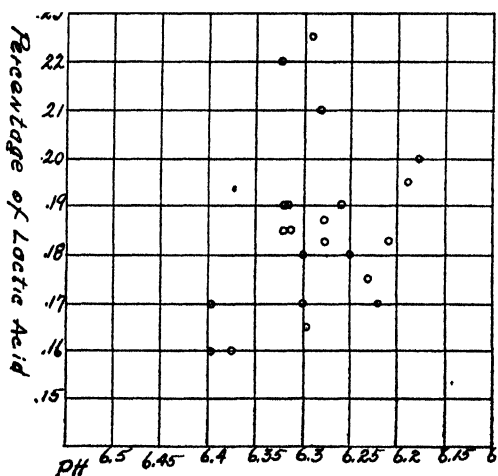
FIG. 3. TITRATABLE ACIDITY AND pH OF MIXES TESTING 12 PER CENT SERUM
SOLIDS

maker, particularly if he standardizes the acidity of the mix in the
manufacturing process.   The hydrogen ion concentration also

was greatest in the mixes of higher serum solid concentration. Table 1 shows the average titratable acidity and hydrogen ion concentration for the mixes used.

That no direct relationship exists between titratable acidity and hydrogen ion concentration may be seen from figures 1, 2 and 3, particularly figure 2.   In this collection may be found several mixes having a titratable acidity of 0.19 per cent but ranging in hydrogen ion concentration from pH 6.119 to 6.41.   At the same time, mixes were found ranging in acidity from 0.15 to 0.21 per cent and having a hydrogen ion concentration of pH 6.3.

The acidity and pH of mixes permitted to develop acid

To study further the relation of titratable acidity and hydrogen ion concentration, several mixes of definite composition were made

TABLE 1

The effect of serum solids on the titratable acidity and pH of mixes

	PERCENTAGE OF SERUM SOLIDS					
	10		11 7		12	
	pH	Acidity	pH	Acidity	pH	Acidity
Highest......	6.42	0 205	6.411	0.21	6 389	0.225
Lowest........	6 16	0 140	6.119	0 15	6 178	0.16
Average.........	6.323	0 1593	6.294	0.1832	6.282	0.1851

and a small amount of lactic culture added after cooling them to 70°F.   At intervals, acidity and pH determinations were made. Three mixes for each composition were studied; the results of these studies are shown in figure 4, lines A, B and C.   A more direct relationship existed between the pH and titratable acidity in the mixes containing developed acid.   While no definite curve could be drawn for the normal mixes, it was found possible to plot a curve with the latter.   It is natural to expect that after the acid has begun to develop, a definite curve could be plotted, as measurements at this time indicate true acidity.   These curves do not coincide with the curve that Sharp and McInerney obtained with fresh and sour milk.   This would not be expected

since the initial acidities of mixes may differ widely from those of samples of milks.   This is due to the difference in composition of milk and ice cream mixes.
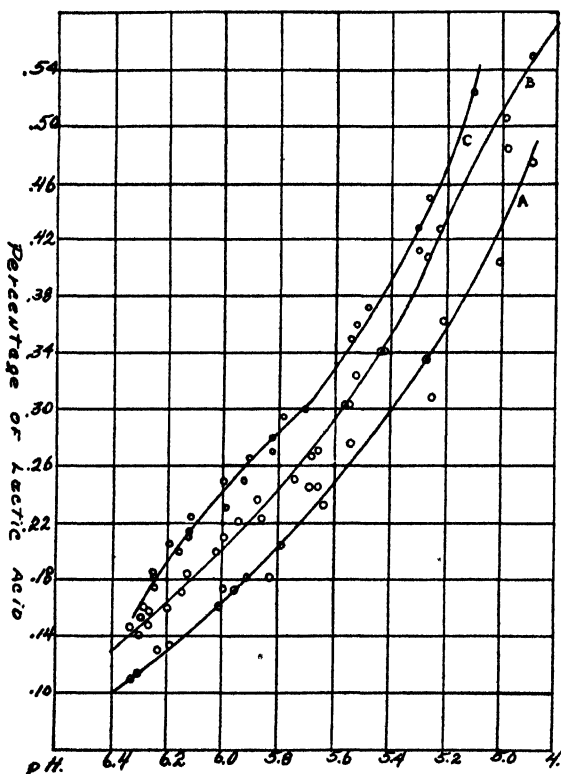
FIG. 4. TITRATABLE ACIDITY AND pH OF MIXES THAT HAVE BEEN PERMITTED TO SOUR

A. Mix containing 8 per cent serum solids.
B. Mix containing 10 per cent serum solids.
C. Mix containing 12 per cent serum solids.

SUMMARY

The titratable acidity of a mix is not a true indication of the amount of lactic acid present.

The figures obtained for titratable acidity are meaningless unless the serum solid content is known.

Knowledge of the serum solid content of a mix, and of its titratable acidity, are not sufficient to enable one to judge the correct point to which the acid may be standardized.

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EFFECT OF A DIET OF SWEET CLOVER ON THE CALCIUM IN THE BLOOD SERUM*

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On account of the splendid value of sweet clover as an aid to soil fertility and also because of its wide adaptability to soil and climatic conditions, its use as a cattle feed has been expanding rather rapidly during the last few years. A demand thus arose to know its feeding value. As a result of this demand some trials were run to compare it with alfalfa and other hays. The majority of the trials point to the conclusion that good sweet clover hay equals alfalfa hay as a source of protein in the dairy cow's ration.

Notwithstanding the high value of sweet clover hay there is a serious reaction against its use because of the numerous deaths in cattle traceable to its use. The first suggestion that deaths in cattle were occasioned by the use of sweet clover was reported by Schofield (14). Up until this·time deaths that may have been caused by sweet clover were attributed to other causes for the symptoms were somewhat confusing.

Later Fitch (2), Mayo (9), Broerman (1), Schalk (13), Graham, McCullock, and Grindley (3), all reported observations where cattle deaths were brought about through the feeding of sweet clover hay. Some of these reports stated that death came because the hay was damaged or moldy. Roderick (11) says "It seems to be definitely limited to sweet clover and only when in the form of spoiled hay or silage."

Probably the most intense study of this disease and its causes is that reported by Schofield (15). His observations led him to the conclusion that this disease was induced by molds that grew on

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the hay. He held that a casual examination would not always disclose the presence of mold, for often times bright appearing hay would show molds in the stems of the plant. He tried feeding rabbits on the hay and found that they responded much as did cattle except that the response was more rapid.

He then attempted to isolate the organism that might be responsible for the reaction in these animals as he is of the opinion that "the theory that the hemorrhage is the result of the delayed clotting time of the blood is not sound," but that a powerful poison is developed which causes degeneration in the walls of blood vessels and capillary endothelium which permits severe ruptures of the blood vessels. Roderick (11) also maintains the theory of toxic injury to the vessel walls.

He also points out that a "prolonged coagulation time and a low percentage of hemoglobin are two conditions frequently observed in connection with this disease whether experimental or natural. The acute form of the disease can occur without either of these changes, but in the sub-acute form it is most likely that both are always present to some extent." He suggests that delayed clotting is "due either to an absence of thrombin or an inhibition of thrombin."

Roderick and Schalk (12) suggest that the best way to tell whether a hay is safe for cattle feeding or not is to feed rabbits on the same hay with the cattle and since the rabbits show the disease symptoms more quickly than do the cattle, one can tell when the hay is unsafe in time to remove the cattle from the feed so as to avoid serious trouble.

As far as we can find no work has been reported which indicates what has happened in the blood of animals which were fed sweet clover hay or silage to cause the blood to lose its power of coagulation. Most writers made suggestions of interference in thrombin formation.

The question arose with us as to whether any interference with the serum calcium had been brought about by the sweet clover ration. As rabbits had been observed by Schofield (15), and Roderick and Schalk (12) to show so clearly the symptoms we wished to study, it was decided to use them as subjects for this

observation in order to determine what changes, if any, take place in their blood-serum calcium while on different diets.

EXPERIMENTAL

The sweet clover (Melilotus alba) used in this trial was gathered during the summer of 1928. It was taken from a heavy stand, growing on a clay soil which was rather rich in alkali.

This feed was gathered by hand with only the finer branches, thickly covered with leaves being taken. These samples were immediately spread on large canvass sheets and exposed to the hot summer sun. The humidity of the air was low which insured a rapid curing. At night the hay was covered by canvass to protect it from any dew. This hay was stored in bags hung in a basement near a furnace so that the temperature of exposure ranged from about 50° to 80°F. Just before the feeding trial this hay was ground to a rather coarse meal.

Three lots of rabbits about three months of age were used in this experiment.

Lot 1 received a diet of sweet clover meal and distilled water.

Lot 2 was placed on a diet of alfalfa meal and distilled water.

Lot 3 received a balanced diet consisting of alfalfa meal, oats, and fresh cabbage or lettuce daily plus water.

Calcium determinations of the serum and weighings of each rabbit were made each week for a period of about two months.

Our method of obtaining the blood from the rabbits was to make a heart puncture with a hypodermic needle attached to a Luer syringe. About 5 cc. of blood were extracted at each time. The blood was allowed to coagulate and was then centrifuged. Two cubic centimeters of the colorless blood-serum were analyzed for calcium by the Clark-Collip Modification of the Kramer-Tisdall Method (5). Calcium was precipitated directly from the serum as an oxalate and titrated with N/100 KMnO₄. (See table 1.)

DISCUSSION

As we had hypothesized the serum calcium of the rabbits on sweet clover fell sharply from the amount carried at the beginning of the trial. It also dropped as compared with the rabbits on

alfalfa and on alfalfa with supplements. This variation downward was from 16 mgm. or above to as low as 12.8 mgm.

The record of rabbit B was inserted because of its interest. It had reached a low point in its serum calcium much faster than other rabbits on the same diet. In an attempt to take the last blood sample, difficulty with the syringe forced a withdrawal of the needle before enough blood for the analysis was obtained. The

TABLE 1

Typical changes of body weight and blood serum calcium of certain of our rabbits

DATE OF BLOOD SAMPLING	DIET						
	Sweet clover meal				Alfalfa meal		Alfalfa meal, oats, cabbage leaves, etc.
	Rabbit A		Rabbit B		Rabbit C		Rabbit D
	Weight	Ca per 100 cc. serum	Weight	Ca per 100 cc serum	Weight	Ca per 100 cc. serum	Weight	Ca per 100 cc. serum
1929	*grams*	*mgm.*	*grams*	*mgm.*	*grams*	*mgm.*	*grams*	*mgm.*
September 11	1,751	16.8	1,688	16.0	1,675	15.9	1,675	15.9
September 18.............	1,542	16.0	1,632	15.8	1,678	15.1	1,768	15.0
October 3........... ...	1,633	13.2	1,724	14.4	1,905	15.8	2,175	15.4
October 10........	1,769	13.0	1,859	12.8	1,996	17.7	2,313	16.4
October 17....	1,769	12.8	1,814	†	2,040	16.0	2,358	15.8
October 24..	1,905	13.6			1,950	15.6	2,493	16.6
October 31................	1,950	12.8			1,950	16.0		‡
November 7.............	1,950	13.0*			2,086	16 0		
November 14..............	2,041	15.2			2,086	16.0		
November 21..............	2,041	15.6			2,086	16.2		
December 6..............		16.1			1,905	16.2		

* Diet of rabbit changed from sweet clover to alfalfa.

† Rabbit bled to death due to slow coagulation before a complete sample could be obtained.

‡ Accidently broke its neck while sample of blood was being taken.

delay was such that before a new attempt at getting more blood could be made, the rabbit showed signs of labored breathing and other trouble. Within ten minutes it was dead.

An autopsy was immediately made. The thoracic cavity was found filled with blood which showed a definitely delayed power of coagulation. We had anticipated that this result would come providing the sweet clover had its usual effect.

Unfortunately, about the time the remainder of the rabbits reached approximately the same level of serum calcium in their blood, our supply of sweet clover had all been used. The rabbits were then changed from sweet clover meal to alfalfa meal with a resultant quick rise in the serum calcium.

At no time did the calcium in the serum of the rabbits on alfalfa meal or alfalfa meal supplemented with other feeds show any tendency to decline in quantity. Evidently this drop in calcium values in this experiment came only under the influence of feeding sweet clover.

An analysis for calcium of the alfalfa and sweet clover used in this trial showed that the alfalfa contained nearly double the amount of calcium that was contained in the sweet clover.

Alfalfa, besides having a large quantity of calcium in its make-up also seems to have a great power in aiding calcium retention by the body as pointed out by Hart and his co-workers (4).

When sweet clover was fed to the rabbits much less calcium was absorbed by their bodies for as has been pointed out (10) "studies of the blood have furnished additional information in regard to the absorption of these particular elements (Ca and P), a rise of concentration in the serum being taken as an indication of increased absorption, and a fall of impaired absorption."

The injurious effect of an insufficient absorption of calcium is more noticeable with growing animals than with mature animals. There may be some correlation here with the observations that "sweet clover poisoning" occurs more often and with a higher death rate among calves and immature animals than among those fully matured.

The question immediately arises as to the effect of this lowered calcium content of the blood on the apparent symptoms in sweet clover feeding, even though it may vary downward to a very considerable extent. The commonly accepted theories (6) of blood coagulation all recognize the essentiality of calcium in activating thrombin. Calcium apparently is not essential in changing fibrinogen to fibrin (6, 7).

The theory put forth by some investigators that thrombin formation is interfered with due to the consumption of sweet

clover hay or silage would necessarily raise the question of the calcium rôle in the delayed clotting of the blood.

Loucks and Scott (7) have pointed out that the blood platelets, which are supposedly the source from which most of the prothrombin originates, do not rupture unless the surface tension of the blood is definitely lowered. From their experiment it seems that anticoagulants prevent the lowering of the blood surface tension by their effect on the blood calcium. They showed also that by an addition of $CaCl_2$ they obtained a definite lowering of the surface tension which allowed clotting through the apparent rupturing of the platelets. They say "Evidently the lowered surface tension produced by adding calcium is the causative factor in the bursting of platelets."

Any lowering, then, of the blood calcium would cause a rise in the surface tension thereby delaying the disintegration of the platelets. Under these conditions small hemorrhages would take place first and as the calcium continued to be lowered the clotting would be interfered with to a greater extent, thereby permitting larger hemorrhages. It has also been observed (15) that there is a lowered quantity of both the red blood cells and the blood platelets as the sweet clover feeding continues.

Our experiment points out that there is a decrease amounting to as much as 25 per cent in the serum calcium of rabbits fed sweet clover hay. The hay we used was evidently of excellent quality as evidenced by the way it was cured and stored and also by the slowness of its reaction on the rabbits. Certain factors produced in sweet clover hay under adverse couring condition perhaps accentuate the calcium loss from the blood thereby accelerating the onset of clot failure, but even without any apparent damage to the sweet clover hay some changes were set up in the blood which ultimately could influence the health of the animals.

The fact that young animals are more susceptible to the "poisoning" than older ones is definitely pointed out and it sustains the contention of calcium interference. In the case of rickets, which is a disease involving calcium metabolism, the young are decidedly more susceptible than are the old, as pointed out by McCollum and Simmonds (8). Even though good hay gives no

apparent trouble when fed, yet surely it does cause some changes in the blood which if continued over a long period might ultimately have its influence on the well-being of the animal. An examination of the weight charts shows that all three of the diets used were able to support regular growth.

SUMMARY

1. There was a decrease in the serum calcium of young rabbits fed on an exclusive diet of sweet clover meal plus distilled water, while it remained fairly constant when rabbits were fed alfalfa meal plus distilled water or alfalfa meal-oats-cabbage-lettuce diets. Upon the resumption of an alfalfa diet the serum calcium content of the blood returned to normal.

2. The decline in serum calcium brought about by sweet clover is probably linked with the failure of the blood to clot in "poisoning."

3. It is suggested that the loss of serum calcium in the blood might raise the surface tension of the blood to such a point that the blood platelets would fail to rupture thus interfering with the formation of thrombin.

4. Rabbits fed on the sweet clover increased their weight at a rate corresponding with those on the other diets.

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THE CALCIUM AND PHOSPHORUS METABOLISM OF HEAVILY MILKING COWS*

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The experiments reported in this paper are part of a mineral feeding project instituted for the purpose of studying the effects of different calcium and phosphorus levels and different mineral supplements on the growth, health, reproduction and milk production of dairy cattle from six months to five years of age.[1]

The investigations of Forbes and his associates (1) indicated that the calcium and usually the phosphorus balances were negative when milk production exceeded 10 pounds per day. Losses of these elements occurred with liberally milking cows regardless of the supply in the ration. Similar results were reported by Hart, Steenbock, Hoppert, Bethke, and Humphrey (2), Meigs, Blatherwick, and Cary (3), and Meigs, Turner, Harding, and Hartman (4). However, Hart, Steenbock, Hoppert and Humphrey (5) stated that they were able to maintain heavily producing cows in positive calcium balance by feeding alfalfa hay cured under caps, and to reduce losses of calcium and phosphorus in cows receiving timothy by the addition of bone meal. Hart and his associates (6) also maintained positive calcium balances by the addition of marl.

Miller and his associates (7) reported increased calcium retention after supplementing the basal ration with bone meal. At the Vermont Agricultural Experiment Station (8) positive calcium and phosphorus balances were maintained when bone meal and limestone rock were added to the ration of liberally milking cows. Perkins and Monroe (9) secured positive calcium balances with liberally milking cows on a narrow ration.

Our own experience indicated that (a) cows could usually be

maintained in positive calcium and phosphorus balance even when producing large amounts of milk and (b) that the needs of the animal determined primarily the amount of calcium and phosphorus that would be utilized. Thus on a low calcium ration in a period of heavy milk production a much higher percentage of the food elements offered in the ration would be assimilated than in a period when no milk was being secreted. Likewise a smaller relative amount, though perhaps a greater absolute quantity, would be utilized from a ration high in calcium and phosphorus than from one poor in these elements.

<div align="center">EXPERIMENTAL</div>

The animals used in this investigation were grade Holsteins. Attention is called to the fact that all were heavy milkers and in some cases unusual ones. Most of them were giving in the neighborhood of 25 kgm. per day at the height of lactation and one, M 253, gave over 35 kgm. per day. Cows M 218, M 234, M 242, and M 281 received a ration consisting of timothy hay, corn silage, and a grain mixture containing 4 parts yellow corn, 2 parts old process linseed oil meal, 1 part oats, and 1 per cent salt after first calving. Beginning at first calving, cows M 222, M 227, M 236, M 240, and M 253 were fed the above ration supplemented with bone flour as 3 per cent of the grain mixture while M 329, M 333, M 334 and M 335 received the same proportion of $CaCO_3$ instead of bone flour. Cows M 214, M 216, M 217, and M 257 were fed alfalfa hay in place of timothy. After first freshening a grain mixture consisting of 4 parts yellow corn, 1 part old process linseed oil meal, 1 part oats, and 1 per cent salt was fed. The three rations were planned to differ only in mineral content, the amounts of protein supplied being about alike. These rations were fed through several years so that the question of the animals adapting themselves to a new diet is not a factor in these results.

The hays used were of unknown history, selected on the basis of color and purity. Most of the timothy used in this work was a high grade No. 2 (United States Standard).

The alfalfa hay used for the main dairy herd was fed to the

TABLE 1

Average daily calcium and phosphorus metabolism of cows fed a basic ration of timothy hay, silage, and grain

ANIMAL NUMBER	PERIOD	Average daily milk	CALCIUM							Ca/P in food	PHOSPHORUS						
			Outgo				Intake	Balance	Used		Outgo				Intake	Balance	Used
			Feces	Urine	Milk	Total					Feces	Urine	Milk	Total			
		kgm.	grams	grams	grams	grams	grams	grams	per cent		grams	grams	grams	grams	grams	grams	per cent
218	Apr. 7–14/27	28.8*	18.76	1.97	27.62	48.35	43.29	−5.06	52.1	0.80	31.92	0.33	21.58	53.83	54.08	0.25	40.4
	Oct. 8–15/28	23.1*	24.31	1.09	23.13	48.53	50.55	2.02	49.8	0.88	23.72	0.08	17.81	41.61	57.12	15.51	58.3
	Aug. 17–24/26	15.8	20.93	2.10	16.76	39.79	40.72	0.93	43.4	0.83	30.64	0.14	14.58	45.36	49.01	3.65	37.2
	Feb. 14–21/27	Dry	13.35	0.91		14.26	26.92	12.66	47.0	1.03	18.17	0.21		18.38	26.24	7.86	30.0
M 234	May 17–24/27	26.7*	17.21	1.76	30.19	49.16	37.38	−11.78	49.3	0.78	28.88	1.30	22.17	52.35	47.71	−4.64	36.7
	Jun. 11–18/28	26.5*	21.46	2.52	30.51	54.49	56.61	2.12	57.6	0.98	30.82	0.16	23.35	54.33	57.96	3.63	46.5
	Dec. 15–22/26	8.0	15.71	1.49	8.07	25.26	30.95	5.69	44.5	1.10	18.26	0.20	6.72	25.17	28.08	2.91	34.3
	Mar. 11–18/28	Dry	16.67	1.04		17.71	27.70	9.99	36.1	1.14	20.43	0.23		20.66	24.20	3.54	14.6
M 242	Aug. 17–24/26	24.8*	28.24	1.48	28.05	57.77	48.92	−8.85	39.2	0.78	43.33	1.65	23.80	68.78	62.99	−5.79	28.6
	Jan. 4–11/27	13.5*	21.67	0.97	14.59	37.23	37.83	0.60	40.2	0.99	26.30	1.53	13.10	40.93	38.32	−2.61	27.4
	Jul. 25–Aug. 1/27	Dry*	27.67	0.74		28.41	34.41	6.00	17.4	1.29	18.71	5.69		24.40	26.67	2.27	8.5
M 281	Jul. 5–12/27	27.6*	25.35	1.29	30.09	56.73	41.34	−15.39	35.6	0.84	30.20	0.22	23.19	53.61	49.30	−4.31	38.3
	Oct. 8–15/28	27.4*	21.40	0.98	27.40	49.78	41.91	−8.87	46.6	0.92	21.97	0.10	21.65	43.72	45.70	1.98	51.7
	Jan. 4–11/27	8.7	13.33	1.07	8.92	23.32	29.90	6.58	51.8	1.04	17.85	0.41	8.30	26.56	28.81	2.25	36.6
	May 17–24/27	Dry	20.17	0.84		21.01	31.94	10.93	34.2	1.38	21.18	0.12		21.30	24.57	3.27	13.3
M 258	Mar. 24–31/28	24.4	29.01	2.67	31.00	62.68	60.18	−2.50	47.4	1.21	28.68	0.10	20.99	49.77	49.66	−0.11	42.0
	Oct. 22–29/28	8.5	21.04	0.77	9.88	31.69	31.44	−0.25	30.6	0.98	20.19	0.10	6.98	27.27	32.02	4.75	36.6

M 259†	Mar. 24–31/28	27.9*	24.93	1.13	33.51	59.57	66.17	6.60	60.6	1.28	22.94	0.12	26.53	49.59	51.51	1.92	55.?
	Oct. 22–29/28	6.8	22.65	0.50	8.01	31.16	29.36	−1.80	21.2	1.04	15.18	0.10	6.04	21.32	28.19	6.87	45.8
M 266†	Apr. 1–8/29	28.2*	28.55	2.16	28.18	58.89	62.02	3.13	50.4	0.81	49.48	0.20	27.06	76.74	76.45	−0.29	35.0
	Mar. 2–9/28	27.5*	27.84	1.29	36.03	65.16	57.71	−7.45	49.5	1.05	29.88	0.08	26	56.64	55.24	−1.40	45.9
	Oct. 22–29/28	1.6	20.58	1.43	2.29	24.30	31.68	7.38	30.5	1.07	16.03	0.12	1.54	17.69	29.72	12.03	45.7

* Not pregnant.
† These animals had pasture in season but were on the basal ration during metabolism.

TABLE 2

Average daily calcium and phosphorus metabolism of cows fed a basic ration of timothy hay, silage, and grain with bone flour as a mineral supplement

ANIMAL NUMBER	PERIOD	Average daily milk (kgm.)	CALCIUM							PHOSPHORUS							
			Outgo				Intake	Balance	Used	Ca/P in food	Outgo				Intake	Balance	Used
			Feces	Urine	Milk	Total					Feces	Urine	Milk	Total			
			grams	grams	grams	grams	grams	grams	per cent		grams	grams	grams	grams	grams	grams	per cent
M 222	Jan. 16–23/28	28.6*	91.99	2.63	36.01	130.63	129.81	−0.82	27.1	1.23	84.85	0.21	27.73	112.79	105.76	−7.03	19.6
	May 18–25/28	21.7	76.01	4.63	24.97	105.61	120.59	14.98	33.1	1.69	41.73	0.22	18.89	60.84	71.58	10.74	41.4
	Apr. 21–28/27	17.6	54.80	4.84	19.49	79.13	88.86	9.73	32.9	1.24	57.05	0.08	16.86	73.99	71.92	−2.57	19.9
	Dec. 26–Jan. 2/29	Dry	52.86	2.27		55.13	64.61	9.48	14.7	1.47	41.87	0.15		42.02	44.12	2.10	4.8
M 237	Feb. 14–21/27	28.7*	86.49	6.65	29.67	122.81	124.14	1.33	25.0	1.20	83.72	0.51	26.33	110.56	103.80	−6.76	18.9
	Aug. 17–24/26	8.9	54.31	2.09	8.90	65.30	70.71	5.41	20.2	1.09	47.14	1.34	8.24	56.72	64.85	8.13	25.2
	Dec. 15–22/26	Dry	37.59	2.15		39.73	53.50	13.77	25.7	1.25	31.01	0.21		31.22	42.84	11.62	27.1
M 236	June 11–18/28	30.3*	96.82	3.12	31.53	131.47	150.40	18.93	33.6	1.47	63.39	0.65	20.92	84.96	102.05	17.09	37.2
	July 5–12/27	23.7*	78.68	3.57	26.77	109.02	104.79	−4.23	21.5	1.15	67.75	3.47	19.43	90.65	91.44	0.79	22.1
	Dec. 17–24/27	9.5	62.03	1.96	11.45	75.44	81.16	5.72	21.2	1.59	42.66	0.01	6.87	49.54	50.89	1.35	16.2
	Apr. 7–14/27	Dry	36.60	1.43		38.03	54.30	16.27	29.9	1.27	34.50	0.10		34.60	42.65	8.05	18.9
M 240	July 5–12/27	23.3*	85.75	2.71	26.60	115.06	106.73	−8.32	17.1	1.12	75.36	0.73	21.46	97.55	95.12	−2.43	20.0
	Oct. 3–10/27	17.1	71.37	3.17	19.71	94.25	99.53	5.28	25.1	1.42	60.20	0.37	15.77	76.34	69.89	−6.45	13.3
	Jan. 4–11/27	8.1	45.16	3.48	9.45	58.09	67.09	9.00	27.5	1.30	42.71	0.50	7.79	51.00	51.67	0.67	16.4
	May 17–24/27	Dry	40.60	1.09		41.69	52.76	11.08	21.0	1.49	34.52	0.22		34.74	35.47	0.73	2.1
M 253	Mar. 11–18/29	36.6*	96.42	1.40	48.29	146.11	148.29	2.18	34.0	1.27	76.57	0.19	36.95	113.71	116.82	3.11	34.3
	Apr. 1–8/29	34.4*	93.81	1.89	42.70	138.40	139.75	1.35	31.5	1.23	77.90	0.21	34.78	112.89	113.36	0.47	31.1
	Mar. 2–9/28	28.1*	103.11	2.63	35.84	141.58	131.22	−10.36	19.4	1.26	85.97	0.09	25.68	111.14	104.01	−7.13	17.8
	Mar. 11–18/27	12.0*	56.66	4.30	12.83	73.79	81.50	7.71	25.2	1.21	56.15	0.21	10.99	67.35	67.09	−0.26	16.0
	Oct. 3–10/27	Dry	54.54	2.42		56.96	63.95	6.99	10.9	1.61	44.97	0.43		45.40	39.63	−5.77	

* Not pregnant.

TABLE 3

Calcium and phosphorus metabolism of cows fed a basic ration of timothy hay, silage and grain with calcium carbonate as a mineral supplement

ANIMAL NUMBER	PERIOD	CALCIUM								Ca:P in food	PHOSPHORUS						
		Average daily milk	Outgo				Intake	Balance	Used		Outgo				Intake	Balance	Used
			Feces	Urine	Milk	Total					Feces	Urine	Milk	Total			
		kgm.	grams	grams	grams	grams	grams	grams	per cent		grams	grams	grams	grams	grams	grams	per cent
M 335	Apr. 1-8/29	28.4*	124.32	0.72	28.13	153.17	173.97	20.80	28.1	2.52	34.14	0.20	27.28	61.62	69.04	7.42	50.2
	Mar. 2-9/28	22.7*	110.84	0.86	24.28	135.98	154.27	18.29	27.5	3.65	29.17	0.08	20.65	49.90	42.21	-7.69	30.7
	Jul. 9-16/28	16.0	103.76	2.00	18.05	123.81	132.47	8.66	20.1	3.18	22.59	0.09	15.97	38.65	41.58	2.93	45.4
	Dec. 26-Jan. 2/29	Dry	51.13	1.14		52.27	65.39	13.12	20.0	2.49	19.86	0.06		19.92	26.21	6.29	24.0
M 329	Jan. 16-23/28	19.4*	100.41	2.18	24.00	126.59	152.15	25.56	32.5	3.52	21.42	0	21.49	43.00	43.16	0.16	50.1
	Jul. 9-16/28	11.5	85.36	4.01	13.75	103.12	113.33	10.21	21.1	3.19	21.02	0.17	11.92	33.11	35.42	2.31	40.1
	Dec. 26-Jan. 2/29	Dry	49.57	1.25		50.82	65.37	14.55	22.2	2.49	21.77	0.13		21.90	26.21	4.31	16.4
M 333	Mar. 24-31/28	18.3*	104.31	1.58	21.83	127.72	154.94	27.22	31.6	3.76	20.45	0.11	17.24	37.80	41.14	3.34	50.0
	Jul. 9-16/28	13.1	84.67	1.98	14.84	101.49	118.68	18.19	27.6	3.18	20.71	0.15	12.35	33.21	37.54	4.33	44.4
M 334	Jun 11-18/28	19.3*	101.83	2.18	23.70	127.71	130.27	2.56	20.1	3.07	25.10	0.23	17.15	42.48	42.37	-0.11	40.2
	Aug. 20-27/29	10.8*	81.15	1.38	13.43	95.96	116.43	20.47	29.1	2.49	30.22	0.40	10.50	41.12	46.74	5.62	34.4

* Not pregnant.

TABLE 4

Average daily calcium and phosphorus metabolism of cows fed alfalfa hay, silage and grain

ANIMAL NUMBER	PERIOD	CALCIUM								Ca/P in food	PHOSPHORUS							
		Average daily milk	Outgo				Intake	Balance	Used		Outgo				Intake	Balance	Used
			Feces	Urine	Milk	Total					Feces	Urine	Milk	Total			
		kgm.	grams	grams	grams	grams	grams	grams	per cent		grams	grams	grams	grams	grams	grams	per cent
M 214	Sept. 13–20/26	20.9*	42.39	0.82	25.87	69.08	73.89	4.81	41.5	1.84	21.03	0.55	18.95	40.53	40.12	−0.41	46.2
	Feb. 14–21/27	13.3	84.89	2.13	13.33	100.35	100.36	0.01	13.3	3.65	15.77	0.19	9.91	25.87	27.44	1.57	41.8
	Jul. 15–22/27	Dry	85.43	1.01		86.44	97.61	11.17	11.4	4.46	17.75	0.08		17.83	21.88	4.13	18.9
M 216	Sept. 13–20/26	27.2*	69.14	1.16	33.58	103.88	106.71	2.83	34.1	2.26	25.69	0.16	24.84	50.69	47.23	−3.46	45.2
M 217	Jan. 16–23/28	28.2*	47.49	1.11	36.47	85.07	85.17	0.10	42.9	1.86	18.67	0.83	27.14	46.63	45.71	−0.92	57.3
	May 18–25/28	17.9	106.26	0.10	20.92	127.28	131.01	3.73	18.8	3.27	22.45	0.31	15.38	38.14	40.06	1.92	43.2
	Apr. 21–28/27	10.0	81.00	0.39	10.64	92.03	94.72	2.69	14.1	3.75	19.58	0.24	10.24	30.06	25.23	−4.83	21.4
	Sept. 13–20/26	Dry	74.67	0.49		75.16	87.99	12.83	14.6	4.33	16.46	0.19		16.65	20.31	3.66	18.0
M 257	Dec. 15–22/26	20.3*	69.87	1.24	23.00	94.11	90.33	−3.78	21.3	1.87	26.32	0.20	20.56	47.08	48.21	1.13	44.9
	Apr. 21–28/27	6.5	76.23	0.61	6.61	83.45	92.45	9.00	16.9	4.49	16.24	0.12	5.57	21.93	20.59	−1.34	20.5
	Oct. 3–10/27	Dry	114.90	1.07		115.97	119.55	3.58	3.0	9.29	11.97	0.27		12.24	12.87	0.63	4.8

* Not pregnant.

animals in this investigation.   No effort was made to procure a
certain grade of alfalfa.   The alfalfa used in this work was usually
lower in grade than the timothy.

Method of procedure

Calcium and phosphorus balances were obtained at various
times throughout the lactation periods and when the cows were
dry.   Seven day collections were made during heavy, medium
or light production, and again during the rest period.   Quantities
of feed were graduated to keep the appetite keen so that all the
food would be cleaned up.   Shavings were used as bedding during
the preliminary period and canvas mattresses during the collection
period.   The metabolism stalls were fitted with special mangers
to prevent loss of feed.   The cows were watered from a tub three
times daily, using college water which contained 0.008 per cent
calcium.   Attendants collected the urine and feces separately by
means of pails and shovels.   The cows were exercised for thirty
minutes each day regardless of weather conditions.

The feces samples were analyzed daily.   The urines and milks
were composited and aliquots, preserved with thymol and formalin
respectively, were saved for analysis. '

The methods of analysis used in this experiment were the same
as in previous work (10).

The average daily milk yields, and average daily calcium and
phosphorus metabolism figures are shown in tables 1, 2, 3 and 4.

The percentage of "calcium used" is the algebraic sum of the
"calcium balance" and the calcium in the milk.   From the stand-
point of the work in hand, milk is considered as a constituent of
the body.   The "phosphorus used" was computed in the same
way.   These correspond to Meigs' values for "assimilation" (4).

DISCUSSION

Calcium and phosphorus balance

We are quite unable to account for the results of Forbes and
his associates (1) which indicated that balances were negative
with cows producing over 5 kgm. of milk per day as our animals

frequently maintained a positive balance while producing from 5 to 7 times that amount. Our results confirm those of Hart and his associates (5) (6) of Perkins and Monroe (9) and of the Vermont Experiment Station (8), all of whom report positive balances with cows giving only slightly less milk than ours. Our results assembled in table 1 indicate that cows receiving a ration of timothy hay, corn silage and grain without a mineral supplement received sufficient calcium and phosphorus for the production of at least 10,000 pounds of milk per year. These cows were on negative balance during heavy production, but later during medium production and when dry stored enough calcium to compensate for the loss during heavy production. It should be emphasized however that in this experiment negative balances or equilibrium was the rule during the first or high production period.

When the basal ration was supplemented by 3 per cent of bone flour or calcium carbonate, positive balances were secured with most cows even during the heavy production period. However, two animals showed negative calcium balances and three negative phosphorus balances in later periods. We are inclined to accept the negative values of the high milking periods as evidence of the failure on the part of the animal to meet the demand of milk production, but subsequent negative balances were probably caused by decreased absorption because of digestive disturbances that prevailed throughout the herd at various times.

The replacement of timothy hay by alfalfa accomplished even more than the addition of the mineral supplement in so far as the calcium balance was concerned. In only one case was there a negative value for calcium balance.

A survey of all these tables reveals the fact that on any of the rations, heavily milking cows were in positive balance most of the time, while on a ration designed to reduce losses, the situation was decidedly favorable for continuous positive utilization of both elements.

Calcium and phosphorus utilization

Meigs and his associates (4) summarized the calcium assimilation figures reported in his experiments and those obtained at

the Wisconsin Experiment Station and concluded "that the average proportion of calcium assimilated by milking cows from fairly well cured hay is somewhere in the neighborhood of 20 per cent of the intake. About the same percentage of calcium is assimilated from timothy hay with its low calcium content as from alfalfa with its much higher one." Our results fail to confirm either of these conclusions. The cows in our experiments receiving timothy hay, corn silage and grain, a ration low in calcium, utilized (with the exception of one low value of 17.4 per cent from 34.2 to 57.6 per cent of the ingested calcium. The one low value was recorded for cow M 242 who failed to conceive and whose need for calcium was therefore reduced. Had she required more for the production of a fetus she would probably have shown a value as high as the others. All cows showed a tendency to increase the assimilation during high productivity when the demands were the greatest. Including all values, the average for the seven cows on this ration were respectively 48.1, 46.9, 32.3, 42.1, 39.0, 40.9 and 40.1 per cent of the food calcium.

When alfalfa was used instead of timothy the calcium intake was more than doubled and the assimilation averages were 22.1, 22.6, 34.1 and 13.7 per cent respectively for the four cows. Individual percentages during the heaviest milking period were much above this as they were in the timothy group. Thus, contrary to Meigs contention, the average proportion assimilated was greatly reduced when calcium-rich alfalfa was used as compared with the proportion assimilated when the calcium-poor timothy was fed.

The groups given the basal ration supplemented with bone flour and calcium carbonate lend further support to our conclusion that the intake and need of the animal determines the proportion of calcium that will be assimilated from the food. In this experiment wherein the intake was greatly increased over that of the basal ration, the averages of calcium assimilated dropped to 27.0, 23.6, 26.6, 22.7, 24.2, 24.0, 25.3, 29.6 and 24.6 per cent.

What has been said of calcium is equally true of phosphorus. The relation between "intake" and "utilization" is particularly well shown in tables 2, 3 and 4. In the experiment concerned

TABLE 5

Ages, stage of lactation and gestation period

ANIMAL NUMBER	DATE OF BIRTH	METABOLISM PERIOD	DATE OF LAST CALVING	DAYS PREGNANT
M 218	Sept. 15/22	Apr. 7–14/27	Feb. 24/27	None
		Oct. 8–15/28	June 17/28	None
		Aug. 17–24/26	Dec. 15/25	76
		Feb. 14–21/27	Dec. 15/25	257
M 234	Apr. 6/23	May 17–24/27	Apr. 21/27	None
		June 11–18/28	Apr. 19/28	None
		Dec. 15–22/26	Apr. 16/26	150
		Mar. 11–18/28	Apr. 21/27	236
M 242	May 17/23	Aug. 17–24/26	July 23/26	None
		Jan. 4–11/27	July 23/26	None
		July 25–Aug. 1/27	July 23/26	None
M 281	June 18/24	July 5–12/27	June 13/27	None
		Oct. 8–15/28	Sept. 8/28	None
		Jan. 4–11/27	June 6/26	133
		May 17–24/27	June 6/26	246
M 258	Dec. 8/23	Mar. 24–31/28	Feb. 11/28	None
		Oct. 22–29/28	Feb. 11/28	172
M 259	Dec. 18/23	Mar. 24–31/28	Feb. 11/28	None
		Oct. 22–29/28	Feb. 11/28	116
M 266	Jan. 12/24	Apr. 1–8/29	Jan. 26/29	None
		Mar. 2–9/28	Jan. 20/28	None
		Oct. 22–29/28	Jan. 20/28	189
M 222	Oct. 14/22	Jan. 16–23/28	Dec. 18/27	None
		May 18–25/28	Dec. 18/27	21
		Apr. 21–28/27	Oct. 18/26	35
		Dec. 26–Jan. 2/29	Dec. 18/27	253
M 227	Dec. 23/22	Feb. 14–21/27	Jan. 9/27	None
		Aug. 17–24/26	Jan. 3/26	133
		Dec. 15–22/26	Jan. 3/26	253
M 236	Apr. 14/23	June 11–18/28	May 9/28	None
		July 5–12/27	May 11/27	None
		Dec. 17–24/27	May 11/27	118
		Apr. 7–14/27	Apr. 3/26	251

TABLE 5—*Concluded*

ANIMAL NUMBER	DATE OF BIRTH	METABOLISM PERIOD	DATE OF LAST CALVING	DAYS PREGNANT
M 240	May 7/23	July 5–12/27	June 7/27	None
		Oct. 3–10/27	June 7/27	None
		Jan. 4–11/27	May 21/26	118
		May 17–24/27	May 21/26	251
M 253	Oct. 19/23	Mar. 11–18/29	Feb. 17/29	None
		Apr. 1–8/29	Feb. 17/29	None
		Mar. 2–9/28	Jan. 26/28	None
		Mar. 11–18/27	Oct. 5/26	None
		Oct. 3–10/27	Oct. 5/26	208
M 335	Mar. 7/26	Apr. 1–8/29	Jan. 20/29	None
		Mar. 2–9/28	Jan. 31/28	None
		July 9–16/28	Jan. 31/28	80
		Dec. 26–Jan. 2/29	Jan. 20/29	250
M 329	Dec. 9/25	Jan. 16–23/28	Dec. 5/27	None
		July 9–16/28	Dec. 5/27	61
		Dec. 26–Jan. 2/29	Dec. 5/27	231
M 333	Feb. 25/26	Mar. 24–31/28	Feb. 4/28	None
		July 9–16/28	Feb. 4/28	62
M 334	Feb. 28/26	June 11–18/28	May 17/28	None
		Aug. 20–27/29	May 21/29	None
M 214	July 28/22	Sept. 13–20/26	Aug. 6/26	None
		Feb. 14–21/27	Aug. 6/26	107
		July 15–22/27	Aug. 6/26	258
M 216	Aug. 16/22	Sept. 13–20/26	Aug. 10/26	None
M 217	Aug. 20/22	Jan. 16–23/28	Nov. 29/27	None
		May 18–25/28	Nov. 29/27	80
		Apr. 21–28/27	Oct. 22/26	61
		Sept. 13–20/26	Sept. 20/25	237
M 257	Nov. 10/23	Dec. 15–22/26	Nov. 20/26	None
		Apr. 21–28/27	Nov. 20/26	78
		Oct. 3–10/27	Nov. 20/26	243

TABLE 6

Composition of feeds

ANIMAL NUMBER	METABOLISM PERIOD	HAY		GRAIN		SILAGE	
		Ca	P	Ca	P	Ca	P
M 218	Apr. 7–14/27	0.510	0.230	0.177	0.400	0.083	0.054
	Oct. 8–15/28	0.317	0.105	0.210	0.479	0.087	0.059
	Aug. 17–24/26	0.303	0.087	0.176	0.434	0.086	0.092
	Feb. 14–21/27	0.277	0.191	0.151	0.375	0.071	0.041
M 234	May 17–24/27	0.320	0.107	0.149	0.399	0.082	0.042
	June 11–18/28	0.336	0.088	0.181	0.438	0.131	0.049
	Dec. 15–22/26	0 290	0.110	0.169	0.394	0.084	0.045
	Mar. 11–18/28	0.320	0.135	0.149	0.400	0.083	0.048
M 242	Aug. 17–24/26	0.303	0.083	0.176	0.434	0.086	0.092
	Jan. 4–11/27	0.272	0.148	0.174	0.399	0.095	0.051
	July 25–Aug. 1/27	0.320	0.116	0.181	0.367	0.128	0.095
M 281	July 5–12/27	0.258	0.126	0.197	0.419	0.069	0.042
	Oct. 8–15/28	0.317	0.105	0.172	0.384	0.087	0 059
	Jan. 4–11/27	0.272	0.148	0.174	0.399	0.095	0.051
	May 17–24/27	0.320	0.107	0.149	0.399	0.082	0 042
M 258	Mar. 24–31/28	0.455	0.091	0.248	0.389	0.100	0 037
	Oct. 22–29/28	0.226	0.149	0.164	0.422	0 097	0.056
M 259	Mar. 24–31/28	0.455	0.091	0.248	0.389	0.100	0.037
	Oct. 22–29/28	0.226	0.149	0.164	0.422	0.097	0.056
M 266	Apr. 1–8/29	0.340	0.139	0.240	0.590	0.123	0 066
	Mar. 2–9/28	0.346	0.142	0.201	0.402	0.111	0.037
	Oct. 22–29/28	0.226	0.149	0.164	0.422	0.097	0.056
M 222	Jan. 16–23/28	0.418	0.137	0.854	0.890	0.191	0.043
	May 18–25/28	0.360	0.082	1.005 ·	0.765	0.118	0.037
	Apr. 21–28/27	0.280	0.126	0.839	0.830	0.105	0.048
	Dec. 26–Jan. 1/29	0.266	0.080	1.010	0.891	0.091	0.057
M 227	Feb. 14–21/27	0.277	0.191	0.830	0.760	0.071	0.041
	Aug. 17–24/26	0.303	0.087	0.844	0.928	0.086	0.092
	Dec. 15–22/26	0.290	0.110	0.825	0.897	0.080	0.045
M 236	June 11–18/28	0.336	0.088	1.030	0.843	0.131	0.049
	July 5–12/27	0.258	0.126	0.863	0.860	0.069	0.042
	Dec. 17–24/27	0.299	0.148	0.840	0.845		
	Apr. 7–14/27	0.510	0.230	0.847	0.865	0.083	0.054

TABLE 6—*Concluded*

ANIMAL NUMBER	METABOLISM PERIOD	HAY		GRAIN		SILAGE	
		Ca	P	Ca	P	Ca	P
M 240	July 5–12/27	0.258	0.126	0.863	0.860	0.069	0.042
	Oct. 3–10/27	0 357	0 123	0.838	0.811	0 130	0.041
	Jan. 4–11/27	0.272	0.148	0.854	0 799	0.095	0.051
	May 17–24/27	0.320	0.107	0.149	0.399	0.082	0.042
M 253	Mar. 11–18/29	0.281	0.156	1.050	0.924	0 117	0 067
	Apr. 1–8/29	0.340	0.139	0.950	0.911	0.123	0 066
	Mar. 2–9/28	0 346	0.142	0.878	0 850	0.111	0.037
	Mar. 11–18/27	0.320	0.135	1.115	0.467	0 164	0 096
	Oct. 3–10/27	0.357	0.123	0.838	0.811	0.130	0.041
M 335	Apr. 1–8/29	0.340	0.139	1.260	0.499	0.123	0.066
	Mar. 2–9/28	0 346	0.142	1 440	0 850	0.111	0 037
	July 9–16/28	0.314	0 089	1.320	0 446	0.151	0 044
	Dec. 26–Jan. 2/29	0.266	0.080	1.130	0.428	0.091	0.057
M 329	Jan. 16–23/28	0 418	0.137	1 310	0.396	0.191	0.043
	July 9–16/28	0 314	0.089	1 320	0.446	0.151	0 044
	Dec. 26–Jan. 2/29	0.266	0.080	1.130	0.428	0 091	0.057
M 333	Mar. 24–31/28	0 455	0.091	1.320	0.362	0.100	0 037
	July 9–16/28	0.314	0.089	1.320	0.446	0.151	0.044
M 334	June 11–18/28	0 336	0.088	1.290	0.435	0.131	0.049
	Aug. 20–27/29	0 386	0.161	1.297	0.490	0.107	0.062
M 214	Sept. 13–20/26	1.651	0.143	0 114	0.362	0.096	0 080
	Feb. 14–21/27	1.412	0.177	0.115	0.315	0 071	0.041
	July 15–22/27	1.175	0.161	0.144	0.335	0.174	0 056
M 216	Sept. 13–20/26	1.651	0.143	0.114	0.362	0.096	0.080
M 217	Jan. 16–23/28	1.580	0.163	0.131	0.340	0.191	0 043
	May 18–25/28	1.793	0.143	0.200	0.429	0.118	0.039
	Apr. 21–28/27	1.270	0.186	0.120	0.341	0.105	0.048
	Sept. 13–20/26	1.651	0.143	0.114	0.362	0.096	0.080
M 257	Dec. 15–22/26	1.428	0 167	0.127	0.337	0 080	0.045
	Apr. 21–28/27	1.270	0.186	0.120	0 341	0.105	0.048
	Oct. 3–10/27	1.740	0.140	No grain		0.130	0.040

in table 2, the basal ration used in experiment I was supplemented with bone flour, causing an increased intake of both calcium and phosphorus. There was a corresponding fall in assimilation values. In experiment IV the substitution of alfalfa for timothy an in experiment III the addition of calcium carbonate increased the calcium intake but left the phosphorus values about as they were in experiment I. There was a marked reduction in the calcium assimilated but the proportion of phosphorus utilized remained practically the same.

The relative importance of total intake of Ca or P and the Ca/P ratio on the amounts of Ca and P used

Many studies have been made on this subject in relation to the production of rickets and malnutrition in growing animals and some work has been done on the importance of the Ca/P ratio in the utilization of these elements by high producing cows (11) (12). In order to get some idea of the relative importance of the total intake of calcium and phosphorus and their ratio in the food data were collected from several sources besides the present paper and treated statistically. A total of 132 experiments were secured from ten different publications (1) (2) (4) (5) (11) (12), embracing animals on various rations in positive or negative balance, pregnant and non-pregnant, and living under different conditions. An arbitrary minimum of 15 kgm. of milk per day was taken to define a heavily milking cow and all the subjects considered were giving this amount of milk or more. The following table shows the indices of correlation.

	INTAKE	Ca/P
Per cent Ca used..........................	0.9053 ±0.0107	0.5704 ±0.0396
Per cent P used...........................	0.8089 ±0.0203	0.3913 ±0.0497

It is evident that the actual intake has a much greater significance than the ratio as regards the utilization of calcium and phosphorus.

SUMMARY

1. A series of calcium and phosphorus balances on cows when in heavy, in medium, or in low milk production and when dry,

on a ration of timothy hay, corn silage, and grain indicates that the ration supplied sufficient calcium and phosphorus for the production of at least 10,000 pounds of milk a year. During the height of production the animals were frequently in negative balance but subsequently positive balances made up the loses.

2. Positive calcium and phosphorus balances were obtained in heavily milking cows when the above ration of timothy hay, corn silage, and grain was supplemented with bone flour. A cow producing 80 pounds of milk a day showed positive calcium and phosphorus balances.

3. Positive calcium balances were obtained in heavily milking cows on a ration of alfalfa, silage and grain.

4. Cows fed a low calcium ration utilized calcium more efficiently than when the ration was high in this element.

5. There was a tendency for cows in heavy milk production to utilize both calcium and phosphorus more efficiently than when in low production or during the dry period.

6. The total intake of calcium or phosphorus has a greater significance in the utilization of these elements than has the Ca/P ratio in the food.

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A STUDY OF THE BACTERIAL CONTENT OF THE FORE MILK OF COWS*

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It has been shown by certain investigators that the fore milk has a higher bacterial count than the middle milk. Little information seems to be available on the number of streams which should constitute the fore milk. This work has, therefore, been outlined with the hope of throwing a little more light on this subject.

Trout (10) using the first three to five streams as the fore milk found an average of 3,900 bacteria per cubic centimeter. The remainder of the milk showed a count of 890 per cubic centimeter. Harding and Wilson (5) reported the average bacterial count based on 1,230 samples from 78 different cows to be 428 bacteria per cubic centimeter. The samples were obtained at the close of the milking. Copeland and Olsen (4) determined the number of bacteria in milk from each quarter of the udder of 40 cows, the samples being taken after approximately one-third of the milk had been drawn. The average number was 1,540 per cubic centimeter. Breed (3) found an average bacterial content of 964 per cubic centimeter for 45 samples of strippings milk drawn directly into sterile test tubes from individual quarters.

In a series of comparisons von Freudenreich (11) found the fore milk averaged 6,000 per cubic centimeter, the middle milk 1,341, and the strippings 769. Stocking (9) using streams 1 and 2; 5 and 6; 9 and 10; 13 and 14 and the strippings showed that in general there is a decrease in the bacterial count as the milking proceeds. Orla-Jensen (7) secured milk in sterile tubes after washing the udder and teats and found the fore milk from 4 quarters contained 16,000 bacteria per cubic centimeter; the middle milk 480, and the strippings 360. Copeland and Olsen (4)

determined the number of bacteria in the fore milk, middle milk, and strippings from each quarter of 8 cows. The fore milk averaged 5,989 per cubic centimeter, the middle milk 577 and the strippings 415.

Russell (8) found that the milk drawn first from each duct contained 2,800 bacteria per cubic centimeter, while the remainder averaged only 330 per cubic centimeter. Work done by Backhaus and Appel (2) showed the germ count to decrease regularly as the milking progressed, and in a few cases, the last portion was sterile. The authors recommended the separation of the first quarter from the last three quarters of the milking. Backhaus and Cronheim (1) used the milk of 8 cows, which was drawn with care into a sterile pail. Rejecting the first 5 spurts of milk, an average bacterial count of 6,600 per cubic centimeter was obtained. Lux (6) did work concerning the total bacterial counts of milk at hour intervals of from 1 to 6 hours, and gave the species of bacteria commonly found.

The present experiment was started October, 1928 and continued through May, 1929. The samples of milk were obtained from the dairy herd at the University of Maryland. The udders of the cows were first washed and dried after which the milk was drawn into sterile Baltimore milk sample bottles. Each sample was a mixture of 4 streams, one being taken from each quarter of the udder. Five such samples were obtained from the first 5 streams and were arbitrarily considered as the fore milk. The next 5 streams from each quarter were discarded and the eleventh stream was used for the sample of middle milk. The samples were placed in the ice box at a temperature of between 40° and 44°F. for 18 hours before plating. The time between drawing the samples and placing them in the ice box was about 15 minutes. The data for the bacterial counts were based on the examination of 100 samples for each stream.

Standard beef extract agar was used as a medium. Recognizing that the medium is not particularly adapted for growing udder bacteria, it would, therefore, not be expected to find as high counts as on the special media. On the other hand, it is believed that in

using standard beef extract agar, a medium is being used which should produce counts comparable with those obtained in standard routine milk analysis.

Dilutions of 1:10, 1:100 and 1:1000 were used in plating the milk. Plating, incubation, and counting were done according to the Standard Methods of Milk Analysis (table 1).

The reduction in bacterial content of stream 11 over stream 5 is so slight that the discarding of the sixth to the tenth streams would seem to be a questionable procedure, from a practical

TABLE 1

Average bacterial counts per cubic centimeter

(Plated on standard beef extract agar)

STREAM NUMBER	AVERAGE BACTERIAL COUNT
1	1,275
2	927
3	705
4	647
5	551
11	449

Average fore milk count was 821 bacteria per cubic centimeter.
Average middle milk count was 449 bacteria per cubic centimeter.

standpoint. The data show that the first 5 streams only may be discarded as the fore milk for the reason that they carry increased numbers of bacteria.

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THE EFFECT OF LIPINS ON THE FAT TEST OF BUTTERMILK*

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INTRODUCTION

As early as 1897 Storch (10) recognized differences in the fat percentage of buttermilk when different testing methods were used. He found that the Rose method gave results 0.04 per cent higher for milk and 0.15 per cent higher for buttermilk than was obtained by drying the samples with kaolin powder and extracting with a Soxhlet apparatus. Mitchell (6) found that buttermilks tested 0.1 to 0.2 per cent by the Babcock method; 0.3 to 0.45 per cent by the rigorous Babcock test and 0.45 to 0.60 by the Roese-Gottlieb method. Lecithin materials have been shown to be present in milk and milk products. Dornic and Daire (3) report 0.0332 per cent of lecithin in buttermilk. Thurston and Peterson (11) working with synthetic milks, to which fat and lecithin were added, concluded that the major portion of the difference between the Babcock and the gravimetric methods of analysis was due to the failure of lecithin to show as fat in the Babcock method. Chapman (1) gives 20.25 as the percentage of the ether extract (in the Mojonnier analysis) that is lecithin. This is equivalent to 0.1302 per cent of the weight of the buttermilk. He further states that when synthetic milks such as were used by Thurston and Peterson (11) were used by him, the percentages of total lecithin added which affected the Babcock, the butyl alcohol and the Mojonnier analyses were respectively 71.0, 68.5 and 76.5 per cent.

Discrepancies among the various methods of analysis of buttermilks do exist. The work so far reported on the effect of lecithin in buttermilk testing has been done on synthetic milks, with the

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exception of Chapman's (1) analyses of the Mojonnier extracts of several dairy products.   It is the purpose of this paper to investigate the extent to which lipins affect the Roese-Gottlieb method of analyzing buttermilk and to offer a tentative hypothesis for discrepancies among the tests not accounted for by lipin.   The material herein presented is but a preliminary report of a study of the nature of the fatty materials found in buttermilk.

Investigation of extraction methods

In order to avoid errors inherent with small quantities of materials, various extraction methods were investigated.   All methods which involved drying the buttermilk before extraction gave weights which accounted for but part of the fatty material extracted by the Roese-Gottlieb method.   An analysis of these extracts gave the percentage of the weight of the buttermilk that was lecithin from 0.005 to 0.0330 per cent.   Continuous wet extraction methods emulsified to so great an extent as to be useless.

The Roese-Gottlieb method, as applied by Thurston and Peterson (11) to 100-gram samples of buttermilk, was finally adopted, with slight modification.   In brief the method used was as follows:

One-hundred-gram samples of buttermilk were weighed into 150-cc. beakers. These samples were transferred to 1-liter separatory funnels. The beakers were rinsed successively with each of the reagents, subsequently added, to insure quantitative transfer; 20 cc. of distilled water followed by 20 cc. concentrated NH_4OH were added and the mixture was shaken thirty seconds; 100 cc. ethyl alcohol were added and the mixture was again shaken thirty seconds; 200 cc. ethyl ether and 200 cc. petroleum ether were added in the order given.   The mixture was shaken one minute after the addition of each of these reagents. When the emulsion had broken, the lower layer was run into a beaker.   The ether-fat layer was run into an 800-cc. beaker.   The buttermilk-water-ammonia-alcohol portion was then returned to the liter separatory funnel.   One-hundred-cubic-centimeter por-

tions of ether and petroleum ether were added successively and the mixture was shaken thirty seconds after the addition of each reagent.   The ether-fat layer from this extraction was combined with the first extract in the 800-cc. beaker.   A pinch of powdered pumice was added to the beaker and the ethers were driven off over a steam hot plate until about 30 or 40 cc. of materials remained. This residue was then transferred through a dry filter into either a fat extraction flask, to determine the weight of the extract, or a

TABLE 1

A comparison of the percentage of fatty materials by the Macro-Roese-Gottlieb, the Roese-Gottlieb, and the butyl alcohol analyses

SAMPLE NUMBER	MACRO-ROESE-GOTTLIEB METHOD	ROESE-GOTTLIEB METHOD	BUTYL ALCOHOL METHOD
	per cent	*per cent*	*per cent*
1	0.62	0.650	0.64
2	0.58	0 636	0.57
3	0.68	0 670	0.72
4	0.64	0.660	0.66
5	0.64	0.610	0.59
6	0 71	0.710	0 74
7	0 78	0 668	0.71
8	0.59	0.600	0.54
9	0.71	0 657	0.61
10	0.77	0.790	0.78
11	0.67	0 670	0.68
12	0.77	0.820	0.76
13	0.77	0.770	0.74
14	0.69	0.690	0 66
15	0.76	0.790	0.62

Kjeldahl flask, for digestion of the extract for the phosphorus analysis to determine the lipin content, or to an iodine flask, for the determination of the iodine absorption value of the extract.

The above method of extraction will be referred to throughout this paper as the Macro Roese-Gottlieb method of analysis. That it is representative of the official method is shown in table 1 which gives a comparison of the "fat" percentage by this method, the Macro Roese-Gottlieb method, and the butyl alcohol method of analysis.

Lecithin which affects the Roese-Gottlieb Fat Analysis of Buttermilk

The fatty extract from 100-gram samples of buttermilk was transferred to 500 cc. Kjeldahl flasks, in petroleum ether solution. The petroleum ether was evaporated and the samples were digested and analyzed by Koch and Woods (4) modification of

TABLE 2

The percentage of distearyl lecithin in buttermilk and the fatty extract of buttermilk
Fatty materials for analysis obtained by the Macro-Roese-Gottlieb extraction.

SAMPLE NUMBER	WEIGHT OF EXTRACT	WEIGHT. DISTEARYL LECITHIN	DISTEARYL LECITHIN IN EXTRACT	DISTEARYL LECITHIN IN BUTTERMILK
	grams	*grams*	*per cent*	*per cent*
1	0.6177	0.1537	25.01	0.154
2	0.5754	0.1475	25.64	0.148
3	0.6701	0.1423	21.83	0.142
4	0.6342	0.1595	25.16	0.159
5	0.6362	0.1406	22.79	0.141
6	0.7066	0.1279	18.54	0.128
7	0.7812	0.1762	22.56	0.176
8	0.6030	0.1482	25.00	0.148
9	0.7066	0.1663	23.53	0.166
10	0.7721	0.1603	20.77	0.160
11	0.6749	0.1491	22.09	0.149
12	0.8240	0.1577	21.30	0.158
13	0.7680	0.1467	19.09	0.147
14	0.6901	0.1028	14.89	0.103
15	0.7573	0.1485	18.73	0.149
Average...............................			21.79	0.149

Neuman's (7) wet digestion method. The phosphorus was precipitated as ammonium phosphomolybdate, dissolved in standard sodium hydroxide, boiled to expel ammonia and titrated with standard hydrochloric acid.

The equation of the reaction under the conditions described is, according to Neuman (7):

$$(2(NH_4)_3PO_4) \cdot (24\ MoO_3) \cdot (4\ HNO_3) + 56\ NaOH \rightarrow$$
$$24\ Na_2MoO_4 + 4\ NaNO_3 + 2\ Na_2HPO_4 + 32\ H_2O + 6\ NH_3$$
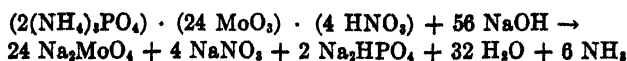
Therefore, one molecule of P_2O_5 or of $Mg_2P_2O_7$ is equivalent to 56 NaOH. From this ratio the distearyl lecithin equivalent to 1 cc. N/1 NaOH can be calculated by the following formula:

$$0.04 \times \frac{Mg_2P_2O_7}{56 \text{ NaOH}} \times 7.27 = 0.02891 \text{ gram distearyl lecithin equivalent to 1 cc.}$$

N/1 NaOH

The results of these analyses are presented in table 2.

The effect of sulfonation of fatty materials in the Babcock test

When the foregoing analyses did not show that the discrepancies among the tests, generally used for buttermilk analysis, were due wholly to lipin bodies, an explanation was sought for that portion of the error not due to lipins. It was considered that, since the fat particles in buttermilk were of extremely small size, the sulfuric acid used in the test would have a large surface for reaction with these fats.

It is well known that sulfonated oils are widely used in the textile industry. Many explanations for the reactions of sulfonation have been offered. Pomerang (8) offers an explanation which, in view of the fact that the rigorous Babcock method accounts for a greater part of the fatty materials of buttermilk than does the regular Babcock method, seems best fitted to the present discussion. He believes that no formula can be offered for the reaction product between the fixed oils and sulfuric acid, but that it is definitely of colloidal nature, and that the reactions are of absorptive nature. Radcliffe and Medofski (9), comparing the constants of the acids of oils before and after sulfonation found in practically all cases, that after sulfonation the iodine values were lower, indicating increased saturation, that the saponification number of the sulfonated oil increases, indicating lactone formation, and that the acetyl values were higher, indicating the formation of hydroxy acids. These authors state that the ease of sulfonation depends not on the degree of unsaturation of the oil but rather on the percentage of olein or oleic acid present. Crowe (2) states that the higher the degree of sulfonation in an oil the greater the solubility of the product in water.

Butterfat contains a high percentage of olein. If the greater part of the sulfonation took place with the unsaturated fats, and caused them to become soluble or to assume a colloidal state which defied centrifugal separation under the usual methods employed in the Babcock test, it seemed logical to investigate the fatty materials of buttermilk to determine whether sulfonation of the olein accounted for that portion of the discrepancy be-

TABLE 3

Relation between the calculated weight of saturated fat (weight of fatty material by gravimetric method less the sum of the lipins, sterols and olein) and the measured Babcock analysis

SAMPLE NUMBER	WEIGHT OF EXTRACT (FROM ROESE-GOTTLIEB ANALYSES)	WEIGHT OF LIPIN IN EXTRACT (AS DI-STEARYL LECITHIN)	WEIGHT OF STEROLS (ASSUMED)	WEIGHT OF OLEIN (CALCU-LATED)	WEIGHT OF SATURATED FAT (CALCU-LATED)	BABCOCK TEST	CALCU-LATED SATURATED FAT LESS MEASURED BABCOCK TEST
	grams	grams	grams	grams	grams	per cent	
1	0.670	0.1503	0.02	0.2171	0.220	0.17	0.050
2	0.670	0.1422	0.02	0.2826	0.225	0.17	0 055
3	0.660	0.1595	0 02	0.2054	0.275	0.21	0.065
4	0.610	0.1447	0.02	0.1881	0 257	0.15	0.107
5	0.710	0.1280	0.02	0.3520	0.210	0.17	0 040
6	0.668	0.1762	0 02	0.2815	0.190	0.20	−0.010
7	0.600	0.1485	0.02	0.2046	0.230	0.34	−0.110
8	0.657	0.1663	0.02	0.1735	0.297	0.32	−0.023
9	0.790	0.1603	0.02	0.2404	0.369	0.41	−0.044
10	0.670	0.1491	0.02	0.1876	0.313	0.28	0.033
11	0.820	0.1577	0.02	0.3483	0.294	0.21	0.084
12	0.770	0.1467	0.02	0.3276	0.256	0.20	0.076
13	0.690	0.1028	0.02	0.2882	0.279	0.13	0.149
14	0.790	0.1485	0.02	0.3363	0.285	0.19	0.095
15	0.730	0.1172	0.02	0.3916	0.201	0.16	0.041

tween the Babcock and the gravimetric methods, not shown by the lipin content. Therefore, the following experiment was undertaken.

Two samples (100 grams each) from each lot of buttermilk analyzed, were extracted as previously described. On one of these extracts the usual Hanus iodine absorption method was used to determine the total weight of iodine, which the extract would absorb. With the other sample, the weight of the extract and the

lipin content (as distearyl licithin) were determined. Four assumptions were then made, one, that the lipin material had one double bond per molecule (Maclean (5)); two, that the unsaturated fats were present as olein, which did not allow for any mixed fats; three, that buttermilk contained 0.02 per cent by weight of sterols (as cholesterol), since the average of the value reported by Thurston and Peterson (11) was 0.0178; and, fourth, that the weight of fatty extract, determined by the gravimetric method, less the sum of the weights of lipins, sterols, and olein, was saturated fat.   The iodine equivalent to the sterol and lipin content was calculated.   This weight of iodine was subtracted from the total iodine abosrbed.   From this difference the weight of olein was calculated.   In the manner indicated above the weight of saturated fat was calculated.   It was considered that the regular Babcock method accounted for saturated fat only, whereas the butyl alcohol and the gravimetric methods measured saturated fats, unsaturated fats, lipin and sterols.   To what extent this hypothesis is substantiated by the experimental data is shown in table 3.

In the above calculations the molecular weight of lecithin was taken as 777.71; the gravimetric factor for iodine equivalent to lipin was $\dfrac{I_2}{\text{Mol. wt. lecithin}}$; that for iodine equivalent to sterols was $\dfrac{I_2}{\text{Mol. wt. cholesterol}}$ and that for olein equivalent to iodine was $\dfrac{\text{Mol. wt. olein}}{3\,I_2}$

SUMMARY AND CONCLUSIONS

When buttermilk is dried before it is extracted the lipin material seems to be destroyed or at least it is not wholly extracted by ether or by petroleum ether from the dried product.   This fact, together with the fact that the average of the lipin analyses reported is 0.149 per cent appears to account for the 0.15 per cent higher fat percentage by the Rose method, than by the Soxhlet method on dried buttermilk which was reported by Storch (10).

The Macro Roese-Gottlieb extraction method used throughout

this work gave weights of fatty material which are in agreement with the Roese-Gottlieb analyses of the buttermilk.

Lipin materials were found in the extracts in quantities ranging from 14.89 to 25.64 per cent of the weight of the extract, with an average of the values 21.79 per cent. The percentages of the weight of the buttermilk that was lipin ranged from 0.103 to 0.176 per cent, with an average of the values 0.149 per cent. These figures are in close agreement with the values reported by Chapman (1), which were 20.25 per cent of the weight of the extract and 0.1302 per cent of the weight of the buttermilk.

The data presented in table 3 would indicate that sulfonation of fats does occur in the Babcock test. They seem to indicate likewise that the error due to sulfonation is at least double that due to lipin materials.

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AIR IN BUTTER*

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The presence of air in butter may have a definite relation to the quality of this product. The variableness of air in butter probably has a direct correlation to weight in making it into prints, for butter is cut into prints by volume.

According to Pickerill and Guthrie (2), the amount of air in butter averages approximately 4 per cent. This agrees with Rahn and Mohr (3), although the range given by Pickerill and Guthrie is wider, varying from 0.5 to 6.0 per cent in one table to 4 to 14.9 per cent in another in which the butter was older.

Recently the author has made several determinations of the amount of air in butter, in a study of the effect of working on the percentage of air. These data will be presented later in this article.

THE METHOD OF DETERMINING THE VOLUME OF AIR IN BUTTER

The amount of air, in the butter in this study, was determined in general by the method of Rahn and Mohr (3). The basis of this method of ascertaining the amount of air is the fact that air is the only component of butter that expands under vacuum. The expansion under vacuum, therefore, is measured and then the amount of air that causes this expansion is calculated by the law of Boyle-Mariotte (1).

One modification on the equipment was made, and one change in the procedure was advantageous. The equipment that was employed in this study is shown in figures 1 and 2. The modification in equipment consisted of the addition of the curved tube in the cap of the glass cylinder. The purpose of this curved tube is to prevent the milkfat that escapes from the metal cone below,

from entering the graduated tube and interfering with accurate reading.

Scale
C.C.
0—

1—

7—

8—

9—

10—
Rubber

Rubber
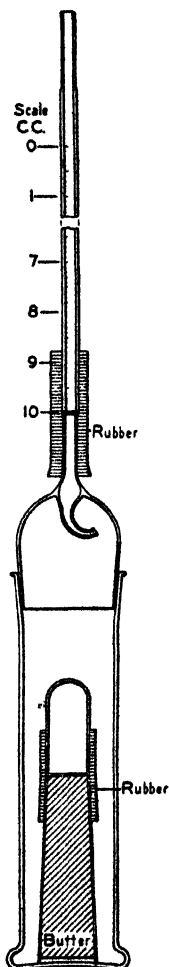
Butter

FIG. 1. THE CYLINDER, THE CONE OF BUTTER, AND THE GRADUATED TUBE IN WHICH THE AIR EXPANSION IS READ

The change in the method is to push the butter up in the metal cone at least $\frac{1}{16}$ of an inch from the lower edge as shown in figure 1. This is accomplished by covering a cork stopper that was approximately the size of the opening in the base of the cone,

with wet parchment paper. The purpose of the wet parchment
is to prevent the sticking of the butter to the stopper. A little
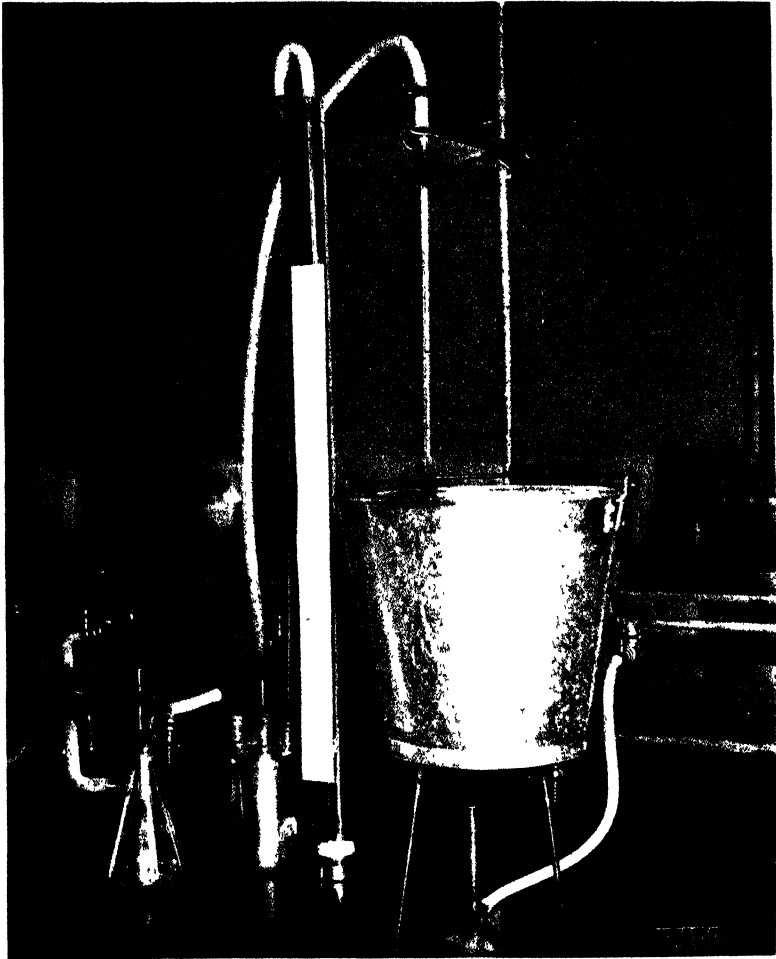
FIG. 2. THE COMPLETE APPARATUS SHOWING THE VACUUM EQUIPMENT; THE
EQUIPMENT IN FIGURE 1 IS IN THE PAIL

pressure is sufficient to force the butter $\frac{1}{16}$ of an inch up the cone.
The purpose of forcing the butter a little way from the lower
edge of the cone is to retain practically all of the milkfat in the

cone, so that only a little of it would flow from under the cone and accumulate in the top of the cylinder.

The cylinder is 4.5 cm. in diameter and 20 cm. long. The dome of the top extends 4 cm. above the main section of the cylinder and the connecting nipple adds about 3 cm. to the entire length of the cylinder, making the over-all length 27 cm.

The cones were obtained from a local tinner and were made of tinned iron. Those of the first set were too narrow at the top, for it was almost impossible to fill them by simply pressing them into the butter. The second set of cones which were filled easily, were 8.2 cm. long, 2.8 cm. in diameter at the base and 2.1 cm. in diameter at the top.

Figure 1 shows the cylinder in which the sample of butter is placed and to which the vacuum is applied. Figure 2 shows the complete apparatus but only the vacuum apparatus is visible because the cylinder, figure 1, is in the pail. Attached to it is the glass tube graduated in 0.1 cc. on which the volume readings are made.

The graduated tube is connected with the glass jar which acts as a trap. The tube containing the mercury is also attached to this trap. It, in turn, is connected with the air line through another trap. The two petcocks which aid in the fine control of the apparatus are directly connected with the latter trap. The centimeter scale on the cardboard is sufficiently large to be easily read. The steps in making the determination are as follows:

1. The metal cones are dried and weighed.

2. The cones are filled by pressing them into a one-pound print of butter. The most satisfactory procedure in filling is to force the cone clear through the print, as it rests, crosswise, on the table. It is necessary that both the top and bottom surfaces of the butter be smooth so that air will not collect in the apertures.

3. The butter is forced at least $\frac{1}{16}$ of an inch up the cone as described above.

4. The metal cones containing the butter, are wiped dry with a clean cloth and then weighed.

5. The glass head, made from a heavy glass test tube, is attached to the small end of the metal cone with a heavy rubber

tube.   This connection is made under the surface of cold water from which the air has been previously driven out by boiling. The temperature of this water must be sufficiently low in temperature to prevent the melting of the butter.

6. The cone with the glass head attached was slipped into the cylinder as shown in figure 1.   This is accomplished under the surface of the water in the pail which may be seen in figure 2. This water must have been previously boiled to expel the air.[1] Then the other connections, as seen in figure 2, were consummated as carefully and as quickly as possible.   In order that no air be caught in the base of the cone, it is essential that the cone be entered in the cylinder in the horizontal position.   The pail, therefore, must be sufficiently large for the cylinder to extend across it with room to spare.   The vacuum was obtained through an air line connected with a Mojonnier tester.

7. In this study, the temperature in the pail ranged from 39° to 49°C.   The exact temperature was always recorded with each set of readings.   A vacuum of 25 mm. was held for five minutes during which time all of the milkfat melted.   A ten-minute period is a little more satisfactory.

8. The first reading on a single cone of butter was always made at 760 mm. (mercury) pressure.   The reading was obtained from the tube as seen in figure 1, which was graduated in 0.1 cc. The succeeding four or five readings were made at steps of decreasing pressures, as read on the mercury manometer.   For illustration the following is a set of readings:

Reading in cc., fig. 1 or 2........	4.1	3.8	3.65	3.15	2.40	1 00
Reading in mm., fig. 2.	760	650	600	500	400	300

9. After the readings in step 8 were taken the vacuum was gradually released and then another set of readings were made on the same cone of butter.   The arrangement of the scale on the gas

[1] If the water in the pail is used too long, small bubbles of air may be observed floating to the top of the cylinder when the vacuum is on.   This is an indication that the water should be reboiled.

burette is such that an expansion of the air in the butter, corresponds to a lower scale reading on the burette. The following data show the readings on this same illustrative determination:

| Readings in cc., figs. 1 or 2................ | 3.90 | 3.60 | 3.20 | 2.60 | 1.65 |
| Readings in mm., fig. 2................ | 760 | 650 | 550 | 450 | 350 |

10. The Boyle-Mariotte law (1) operates according to the equation $V_0 P_0 = V_1 P_1$.

V_0 is the unknown volume X at P_0 or atmospheric pressure of 760 mm.

TABLE 1

Calculating the volume of air in butter

$760 - P$	P	READING IN GRADUATED TUBE	Dv	X
	760	4.1		
110	650	3.8	0.3	1.772
160	600	3.65	0.45	1.687
260	500	3.15	0.95	1.826
360	400	2.40	1.70	1.888
460	300	1.00	3.10	2.021
Average..				1.839

V_1 is the volume at the decreased pressure P_1

$V_1 = X +$ the measured increase of the volume or

$V_1 = X + dv$

$$X \cdot 760 = (X + dv) P_1$$
$$X \cdot 760 = XP_1 + dv\, P_1$$
$$X (760 - P_1) = dv\, P_1$$
$$X \quad \frac{dv\, P_1}{760 - P_1}$$

The application of these equations are made directly in table 1, to data in this study. In this table the heading "760 − P" is 760 mm., atmospheric pressure minus the pressure at which the reading was taken. "P" is the pressure of the air in the butter,

at which the reading was made. "Reading in graduated tube" is the reading on the tube, as seen in figure 1. "Dv" is the difference between the reading on the graduated tube at 760 mm. and the one immediately concerned. "X" is the unknown volume at atmospheric pressure of 760 mm.

11. The next calculation is to correct for temperatures. The air content of butter is always measured at melting temperatures. The volume occupied by water vapor at the various temperatures must be considered. The saturated pressure of water vapor at 40°C. is 55 mm. mercury or 55/760 of the air space calculated for 760 mm. or atmospheric pressure.

TABLE 2

Calculating factors for different temperatures

VAPOR PRESSURE	TEMPERATURE	FACTOR	VAPOR PRESSURE	TEMPERATURE	FACTOR
mm.	*°C.*		*mm.*	*°C.*	
49	38	0.875	71	45	0.848
52	39	0.872	75	46	0.843
55	40	0.868	79	47	0.838
58	41	0.864	83	48	0.833
61	42	0.861	87	49	0.829
64	43	0.858	92	50	0.823
68	44	0.852			

As an example of calculation for 40°C.

$$V_{40} \quad \frac{X \,(760 - 55)}{760} \quad \frac{X \,705}{760}$$

The volume was further corrected to a standard temperature of 40°C.

$$V_{20} = V_t \frac{273 + 20}{273 + t}$$

The following is an example of changing the volume at 40°C. to the volume at 20°C.

$$V_{20} = V_{40} \frac{273 + 20}{273 + 40} = \frac{X \,705 \cdot 273 + 20}{760 \cdot 273 + 40} = X \, 0.868$$

This corrective factor, 0.868, must be applied to the average reading in column "*X*" of table 1 to bring 40°C. to the saturated-pressure of 20°C. The following table contains the corrective factor and vapor-pressure for the different temperatures.

TABLE 3

The effect of working butter on the percentage of incorporated air

SAMPLE	PRINT 1		PRINT 3		PRINT 5		PRINT 7		PRINT 9		PRINT 11	
	First reading	Second reading	First reading	Second reading	First reading	Second reading	First reading	Second reading	First reading	Second reading	First reading	Second reading
Section 1:												
a.........	6.008	5.453	4.552	4.991	3.287	3.059	3.522	3.600	3.547	4.602	5.635	5.718
b.........	6.660	6.671	4.313	4.674	2.181	2.382	3.658	3.835	5.328	5.626	5.176	5.399
c.........	7.026	6.860	6.450	6.383	2.579	2.509	4.186	4.118	4.965	5.560	5.432	6.274
d.........	6.168	6.660	4.169	4.332	4.590	4.307	4.272	4.161	5.005	4.612	4.516	4.654
e.........	6.648	6.351	3.260	3.633	2.797	2.392	3.658	3.731	4.887	5.149	5.122	5.302
f.........	6.021	5.710	4.214	4.533	2.343	2.476	4.042	4.064	5.065	5.242	4.140	4.429
Average ...	6.421	6.284	4.493	4.758	2.962	2.854	3.889	3.918	4.799	5.131	5.003	5.295

	PRINT 2		PRINT 4		PRINT 6		PRINT 8		PRINT 10		PRINT 12	
Section 2:												
a.........	7.390	7.081	5.307	5.002	5.111	5.368	4.932	4.932	4.636	4.630	5.242	5.592
b.........	6.713	6.602	5.578	6.008	5.151	5.125	2.846	3.887	3.801	4.196	5.063	5.029
c.........	3.615	3.595	4.188	4.612	4.844	4.780	4.384	4.586	4.348	4.381	5.820	6.314
d.........	6.310	6.595	6.348	6.221	5.966	5.212	5.765	5.718	2.603	2.912	6.301	6.317
e.........	6.260	6.533	4.877	4.944	6.193	5.904	5.735	5.584	3.971	4.077	6.076	6.395
f.........			4.392	4.269	6.090	5.793	5.246	4.882	4.115	3.929	6.008	6.240
Average....	6.668	6.703	5.115	5.176	5.559	5.363	4.817	4.931	4.174	4.243	5.752	5.981

The grand average of the fairly well worked butter of section 1 was 4.651 per cent air, while the grand average of the thoroughly worked butter of section 2, was 5.374 per cent air. This is a difference of 0.723 per cent.

12. The final computation of the percentage of air in butter follows this equation,

$$\frac{X \cdot F \cdot 100}{Wt} \quad X = 1.8$$

as noted in table 1. F = 0.852 which is the temperature correction factor at 44°F., as seen in table 2. The 100 is used to con-

vert the quotient into percentage. And Wt = the weight of the butter which was 36.1542 grams.

$$\frac{1.839 \cdot 0.852 \cdot 100}{36.1542} = 4.333 \text{ per cent}$$

In like manner the calculation of the volume of air in terms of percentage may be obtained from the second set of readings which are recorded in step 9.

THE RELATION OF THE AMOUNT OF WORKING THAT BUTTER RECEIVES TO THE AMOUNT OF INCORPORATED AIR

The data in this study of the relation of the amount of working that butter receives to the mount of air incorporated in it, may be seen in table 3. The first section of this table gives figures on butter that was worked sufficiently to prevent the formation of mottles. This butter contained fairly large droplets of incorporated water. The second section presents figures on the same lots of butter that were thoroughly worked. This latter butter appeared dry, for the moisture droplets were very small.

The butter was made in a combined churn and worked in 6 different churnings. The prints, weighing 1 pound, were taken in the following manner; print number 1, was obtained from the middle of the churn when the butter was fairly well worked; print number 2, was taken from the same location in the same churning after the butter was thoroughly worked. In like manner, the remaining pairs of prints were obtained from the churn when the butter was fairly well worked, and when it was thoroughly worked.

The samples (a, b, c, d, e, f) are the conefuls that were taken from each print. The columns that are headed "First reading" or "Second reading" give data on the first series of readings after the vacuum was applied; and then on the second series after the vacuum was released, and again applied to the same sample of butter. Each single reading for each sample of butter is an average of 5 or 6 individual sets of figures. Both readings of sample c in print 2, and sample d in print 10 were omitted for they were much out of line.

Table 3 shows an average increase of air content in the butter, due to working of 0.701 per cent. The lowest increase was 0.227 per cent as found in the first readings of prints 1 and 2. The highest increase was 2.521 per cent as seen in the first readings of prints 5 and 6. Prints 9 and 10 reserved the figures and gave a decided decrease even though the two readings of sample d were omitted.

These data which show a little greater variation than that found indicate that one effect by Rogers (4), of working butter is to slighly increase the percentage of incorporated air. Under certain conditions, the variation in air content between the butter that is fairly well worked and that which is thoroughly worked, may be sufficient to materially affect the procedure of packing butter, particularly of printing it; for print butter is packed by volume and sold by weight.

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A NEW FORM OF LACTOSE CRYSTAL FOUND IN SANDY ICE CREAM*

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In contrast with the lactose crystals usually found in sandy ice cream, originally described by Zoller and Williams (1) as lactose hydrate ($C_{12}H_{22}O_{11} \cdot H_2O$) and characterized by a distinct tomahawk appearance, another form of crystalline milk sugar has been found in an investigation conducted in these laboratories. This type of crystal is diamond in shape and may develop in ice cream when produced and held under certain conditions.

These crystals which form at a slow rate, and apparently from only very viscous solutions, have likewise been proven to be lactose hydrate.

Since this type of crystal had not heretofore been observed in ice cream, there was some doubt with respect to its preliminary identification; its unusual shape suggested the possibilities that it might be either the beta (β) form of lactose, or sucrose in the early stage of development.

The new crystals were first examined crystallographically and later chemical and other physical tests for their identification were made.

DETECTION OF NEW FORM OF CRYSTAL IN ICE CREAM

The diamond shaped crystal was first found in ice cream that contained 12 per cent butterfat, 16 per cent sugar, 12 per cent serum solids, and 0.3 per cent gelatin. The unusual shape of this crystal was discovered while making a microscopic and photographic study of grease covered slides (fig. 1), designed by the authors for the purpose of determining and recording the degree of texture variations occurring in ice cream. The ice cream had been held in the hardening room about five weeks

when the sandy texture was first observed. The "sand" had developed in three cans of ice cream and after the new form of crystal had been noticed it was possible to separate a relatively large quantity for chemical and microscopic study. A considerable portion of these crystals has been saved for future reference.

<center>SEPARATION AND IDENTIFICATION</center>

The crystals were removed from the ice cream by first centrifuging the melted product in large glass tubes. The fat and other liquid were then removed by decantation and the crystals obtained (fig. 2) were washed by shaking with a slightly ammoniacal solution of equal parts of alcohol and water which had been cooled to $-5°C.$, the solution being made slightly alkaline in order to dissolve the precipitated and denatured proteins. This procedure was repeated several times to insure the complete removal of the protein from the surface of the crystals and, after a final washing with ether, clean crystals were obtained (fig. 3).

The crystals were dried at room temperatures and were submitted to Dr. Edgar T. Wherry of the Bureau of Chemistry and Soils for identification. These crystals proved to be too small to permit a full description of their crystallographic characteristics; furthermore they could not be compared with all possible forms of lactose since at that time there appeared to be no information in the literature concerning the crystallography and optical properties of β-lactose (2). It was necessary therefore to prepare several samples of this type of crystal (fig. 4) for more complete microscopic measurements and for chemical examination.

A preliminary crystallographic examination showed that these diamond shaped crystals had the refractometric characteristics of alpha (α) lactose hydrate.

<center>CHEMICAL AND PHYSICAL EXAMINATION</center>

The reduction test with Fehling's solution proved conclusively that the new crystals were not sucrose; this fact and their occurrence in a relatively large quantity showed that they were probably some form of lactose.

A series of melting point determinations of the diamond shaped

crystals were made and compared with this property of α-lactose hydrate and β-lactose anhydride. The results of these tests are shown in table 1.

The melting point determinations, made upon three separate samples of β-lactose anhydride crystals prepared in these laboratories, were in reasonable agreement with the melting point given by Gillis; and, melting point determinations made on three

TABLE 1

Melting point determinations of lactose and sucrose crystals

	MELTING POINT*
	°C
β-lactose anhydride	
Leighton, prepared by	252 9
Williams, prepared by	252 4
Williams, (large crystals) . ..	253 4
Gillis (3)	252 2
α-lactose hydrate·	
Diamond shaped crystals	199 3
Diamond shaped crystals	201 1
Diamond shaped crystals	199 8
Gillis (3) . .	201 6
α-lactose anhydride:	
Gillis (3) .	222 8
Sucrose (different observers) .	160-180

* Melting point tubes containing the sample were immersed beside the thermometer bulb for 5 seconds. The bath used was a NaNO₃-K₂CO₃ fusion mixture Anschutz thermometers (Bureau of Standards calibrated) were used.

samples of the diamond shaped crystals agreed closely with the melting point given for the α-hydrate. These results proved conclusively (a) that the new crystals were not β-anhydride or α-anhydride lactose, and (b) that they were α-lactose hydrate but a crystal form which heretofore had not been found in sandy ice cream.

The melting points of α-anhydride lactose and sucrose are also shown in the table.

CONDITIONS CAUSING FORMATION OF NEW CRYSTAL FORM OF LACTOSE

The formation of the ordinary type of "sand" (fig. 6) in ice cream is usually attributed to one or more of the following factors: (a) a high concentration of lactose, (b) insufficiently rapid freezing, (c) temperature fluctuations in hardening rooms and
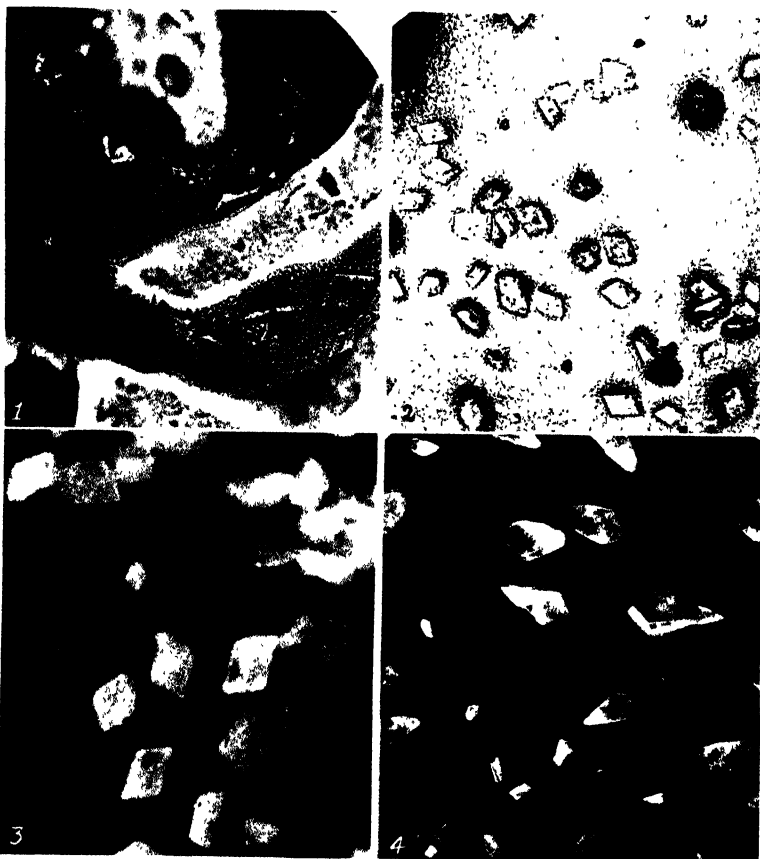
FIG. 1. DIAMOND SHAPED CRYSTALS IMBEDDED IN GREASE COVERED SLIDE

FIG. 3. DIAMOND SHAPED CRYSTALS WASHED FREE OF PROTEIN

FIG. 2. DIAMOND SHAPED CRYSTALS WITH COATING OF PROTEIN MATERIAL

FIG. 4. β-LACTOSE ANHYDRIDE CRYSTALS

(Natural size)

cabinets, and (d) the presence of crystal and other nuclei such as nuts, fruit seeds, etc.

FIG. 5. DIAMOND SHAPED CRYSTALS FOUND IN SANDY SWEETENED CONDENSED MILK. × 130 (Hunziker and Nissen)

FIG. 7. "SAND" CRYSTALS FROM CONDENSED WHEY. × 90 (Zoller and Williams)

FIG. 6. "SAND" CRYSTALS FROM ICE CREAM. × 90 (Zoller and Williams)

FIG. 8. CRYSTALS OF PURE SUCROSE (Natural size)

The formation of the new type of lactose in ice cream is probably due to the first three mentioned factors and its crystal form, in particular, is likely caused by the high concentration of sucrose.

Thus Hunziker and Nissen (5) have found that while the colloids in milk do not have any material influence on the solubility of lactose, the presence of sucrose does diminish the solubility of milk sugar, and that a high sucrose concentration in lactose solutions also has a very noticeable influence on lactose crystal formation, the shape of the lactose crystal being distinctly modified.

Since these investigators have also shown (4) that "the presence of milk colloids in saturated lactose solutions does in no way interfere with the full development of the lactose crystals" and that diamond shaped crystals are formed in sweetened condensed milk (fig. 5) the occurrence of similarly shaped crystals in ice cream can be attributed to the high concentration of the sucrose in the solution, mainly caused by freezing and separation of a large part of the water. It appears probable that the more viscous the solution from which lactose crystallizes, the more likely is the crystal form to be modified. If, however, the ice cream be maintained at such a high viscosity (frozen hard, or approximately so) as to prevent diffusion, separation of the lactose, if it takes place at all, will result in the formation of minute crystals. Such crystals, or very fine "sand," are practically undetectable when this product is consumed and hence are not objectionable.

SUMMARY

Diamond shaped crystals occurring in "sandy" ice cream have been identified as α-lactose hydrate.

It is believed that the occurrence of this modified form of lactose crystal is caused by the high viscosity of the solution due in particular to the concentration of the sucrose in the crystallizing medium, which in turn is caused by a freezing out of part of the water.

The relatively large size of the diamond shaped crystals, as well as their quantity, indicates that, for the avoidance of "sand" in ice cream, the viscosity of the solution, in addition to the solubility relationships of lactose, must be considered.

It is recommended that ice cream be kept frozen hard or approximately so, thus preventing diffusion and the growth of relatively large lactose crystals or objectionable "sand."

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COTTONSEED MEAL STUDIES

III. HEAVY FEEDING OF COTTONSEED MEAL TO DAIRY CATTLE DURING REPRODUCTION AND LACTATION*

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Cottonseed meal usually furnishes the cheapest available source of a high protein concentrate but it is not heartily recommended by all authorities. It is seldom recommended as a feed for calves and only in small amounts for pregnant or lactating cows. If fed heavily, it is thought to produce injury. Cottonseed meal injury has been attributed to beri-beri, pyrophosphoric acid, bacteria and molds, gossypol, betaine and choline, iron deficiency, protein decomposition products, acidosis, and lack of a factor or factors carried by hay. A lack of a factor or factors carried by hay seems very probable in view of the fact that in sections where cottonseed meal is produced and therefore available, cottonseed hulls have been used largely as a roughage. Cottonseed hulls probably lack a factor or factors carried by a good quality of hay.

The purpose of this investigation was to study the effects of cottonseed meal as the principle source of protein for growth, reproduction, and lactation over a long period of time with a ration otherwise adequate.

This is the third of a series of papers on cottonseed meal to be reported from the Dairy Section of the Michigan Agricultural Experiment Station. It was shown in a previous report (1) that the liberal feeding of cottonseed meal to growing heifers along with timothy hay and corn silage, did not produce the symptoms of cottonseed meal injury. However, when cottonseed meal was fed with wheat straw as a roughage, the symptoms of cottonseed meal injury were manifested.

The present paper is a progress report giving the effect of heavy

cottonseed meal feeding on health and reproduction of the first generation of dairy cattle to about four years of age.

Moore (2) investigated the effect of heavy feeding of cottonseed meal on health and reproduction of dairy cattle. Three lots of 5 animals each were used. The animals in lot I were fed heavily on cottonseed meal with a little other grain and received hay and silage principally as a roughage. Those in lot II received a grain ration containing no cottonseed meal but with cottonseed hulls principally as a roughage, while those in lot III received a good grain ration with hay and silage principally as a roughage, but no cottonseed products.

Milk production and breeding data were given as follows:

	MILK PRODUCTION FIRST 180 WEEKS	TIMES BRED	CALVES DROPPED	MONTHS BETWEEN CALVING	CASES OF GARGET
Lot I	13 7	56	22	14	14
Lot II	14 3	41	24	13	1
Lot III	11.7	29	24	12	2

In lot I, one cow lost two quarters of her udder and two cows lost one quarter each, while in lot II, one cow lost one quarter of her udder. In lot I there were three cases of retained afterbirth, and in lot II one case of abortion. One calf was born dead in lot I, and another was weak.

Moore concluded from these results that the feeding of 5 pounds of cottonseed meal per day for any great length of time is injurious to the dairy cow, causing inflammation of the udder, difficult breeding and retention of the afterbirth, although apparently no symptoms of cottonseed meal poisoning were noted.

Combs and Curtis (3) of the North Carolina Station fed 5 lots of 5 animals each cottonseed meal at the rate of one pound daily per 100 pounds live weight. Cottonseed hulls, beet pulp, and corn silage in various combinations were fed as roughage. Blindness, weakness, abortion, dead and weak calves occurred. Sev-

eral of the animals died, but no deaths occurred in the lot receiving corn silage.

The work of Combs and Curtis has been continued at the North Carolina Experiment Station by them and several other investigators to the present time.

In 1923 (4) these investigators reported that the previous rations were supplemented with 5 to 10 per cent alfalfa meal, with the result that two living calves were prodced and convulsions did not occur.

In 1924 (5) mineral salts added to the ration of cows whose roughage was cottonseed hulls, and grain made up entirely of cottonseed meal, were of no benefit. Calves were born prematurely, and even though some gestation periods were normal, the calves were weak. The addition of small amounts of other supplements which were described in this report as "certain other substances" proved to be highly beneficial. The following statement is made:

Where cottonseed meal and hulls have been supplemented liberally with corn silage and cracked corn, the cows receiving this ration have not been able to give birth to normal calves and milk normally. But with the addition of small quantities of certain other substances to the rations, these cows have given normal calves and have milked well above the state average.

In 1925 (6) a very interesting fact was mentioned as follows:

Approximately five years ago twenty head of cattle on heavy cottonseed meal feeding, kept on a lot of four acres of ground, produced apparently normal living calves. In this lot these cows had access to small amounts of grass which they kept clipped short. This herd was later moved to a small closely fenced lot where no green grass was obtained with the astonishing result that abortions, dead and underweight calves, and living blind calves were obtained.

In 1926 (7) four lots of cows which were fed a concentrate ration of from 50 to 100 per cent cottonseed meal with a good roughage reproduced normally. They further reported that during 1926 four groups of 3 animals each were placed on an experiment with wheat straw as roughage and using as concentrates in lot I,

cottonseed meal; lot II, linseed oil meal; lot III, peanut meal, and in lot IV, soy bean meal. All animals received minerals.

In 1927 (8) the roughage was changed in the above lots to beet pulp and cottonseed hulls. All animals in all groups reacted similarly.

One animal out of the first 3 lots and all out of lot IV died. The addition of cod liver oil alleviated the deficiency symptoms of the remaining animals.

In another group of cows, those animals receiving the poorest quality of roughage failed to respond as did those where alfalfa hay was used.

Bell and Williams (9) reported that cottonseed meal fed at the rate of one pound per day to 1000 pounds live weight to mares during the period of pregnancy showed no ill effects from its consumption, nor were any ill effects noticeable on the colts when foaled, nor did the feeding of cottonseed meal prevent the mares from becoming pregnant.

Gray and Ridgeway (10) fed 65 pregnant ewes from 0.2 to 0.8 pound of cottonseed meal for varying periods of 35 to 210 days with no ill effects except in one case where blindness and death occurred. During the four years the work was carried on 6 cases of abortion occurred among the ewes eating cottonseed meal but there were as many abortions among the ewes in the check lot which received no cottonseed meal.

The Texas Station (11) reported that 5 brood sows receiving a ration containing 15 per cent cottonseed meal seemed to stay in a good condition and produced as many pigs as did the tankage fed sows but the pigs did not grow quite as rapidly as did the tankage fed pigs.

Richardson and Green (12) found that a ration containing 50 per cent cottonseed flour, protein free milk and butterfat was sufficient for normal growth and development and reproduction to the third generation.

EXPERIMENTAL

This experiment was begun in 1926 in order to determine the effect of heavy feeding of cottonseed meal with ample roughage of

high quality on growth, health, reproduction, and milk production of dairy cattle over a period of three generations. The effects of cottonseed meal feeding on growth and health of the first generation from 3 to 18 months have been previously reported (1), and also the effect of heavy feeding of cottonseed meal on the consistency of feces of dairy cattle (13). The present paper reports the effect of heavy feeding of cottonseed meal on health, reproduction, and milk production of dairy cattle for the first generation up to approximately four years of age. The first generation of animals used in this investigation consisted of 10 high grade Holstein heifers. They were divided into 2 lots of 5 animals each.

Animals in lot I, G-1, G-3, G-5, G-7, and G-9, received cottonseed meal as the principal source of protein, whereas in lot II, G-2, G-4, G-6, G-8, and G-10, received linseed oil meal as the principal source of protein. Linseed oil meal was fed as a check against cottonseed meal since the former is recognized as a safe protein concentrate.

The inheritance of the animals in both lots was similar. Eight of the animals, G-3 to G-10 inclusive, were sired by the same bull. Animal G-1 and the dam of G-10 were also sired by the same bull. The dams of G-3, G-4, G-5, and G-6 were sired by the same bull as well as the dams of G-7, G-8, and G-9. Animals G-7 and G-8 are twins. The milk records of the dams of the animals varied from 7,343 pounds to 9,440 pounds.

Rations

The cottonseed meal used in this investigation was purchased on the open market. Up to the first calving time the animals in lot I received sufficient cottonseed meal in the ration to meet the Armsby requirements for protein. Since a slightly smaller amount of cottonseed meal was fed than linseed meal in order to get the same amount of protein, a small amount of yellow corn was fed to lot I to bring the total digestible nutrients up to the same level as contained in the larger amount of linseed oil meal. Corn silage and good quality timothy hay were fed as roughage in whatever amounts the animals would consume. The timothy hay used was high grade No. 2 as to color and No. 1 as to purity. The pro-

tein in the corn, corn silage, and timothy hay was considered as excess protein.   The animals in lot II were fed similar to those in lot I except old process linseed oil meal replaced the cottonseed meal in the ration.   Two per cent bone meal and one per cent salt was added to the protein concentrates fed.

After first calving the animals were fed similary except the requirements for maintenance and milk production were calculated according to the upper limits of the Morrison Feeding Standard.
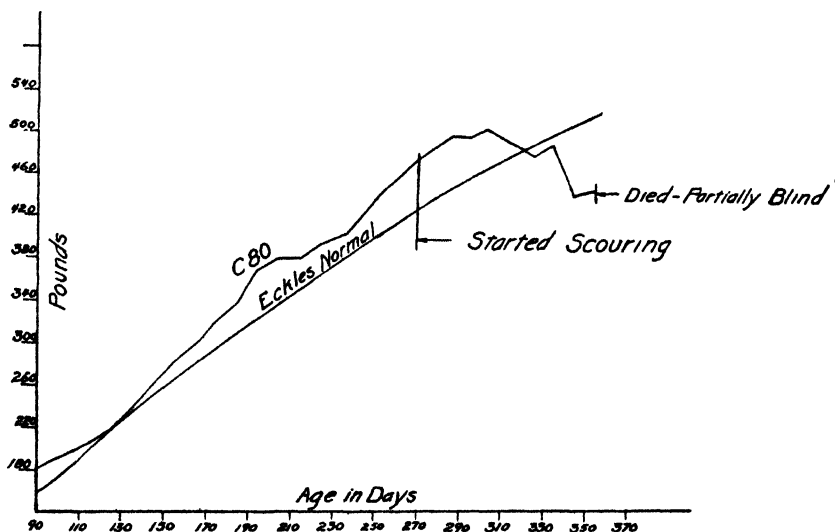
Fig. 1. Showing Effect of the Cottonseed Meal Used in This Investigation on Growth and Health of C-80 When Fed with Roughage of Poor Quality (Wheat Straw)

In cases where the cottonseed meal or linseed meal, silage, and timothy hay consumed did not furnish sufficient total digestible nutrients, the deficiency was made up with ground yellow corn.

Production of cottonseed meal injury

The cottonseed meal used in this investigation was the same as that fed to calf C-80.   This Holstein bull calf was placed on a ration of cottonseed meal, wheat straw, corn, oats, bone meal, and salt at 94 days of age.   Skim milk was also fed until 150 days of

age. Figure 1 shows the growth in weight of this animal compared to Eckles' normal (14).

Food consumption is shown in table 1. This animal died at 361 days of age with the characteristic symptoms of cottonseed meal

TABLE 1

Showing feed record of calf C-80, which was fed cottonseed meal from the same shipment as was used in this investigation

10-DAY PERIODS	SKIMMILK	COTTONSEED MEAL	CORN	OATS	WHEAT STRAW
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
1	120	3.7	9 5	9.5	12 0
2	120	9.0	5.0	5 0	10 0
3	120	14.6	5.0	5 0	13.0
4	120	20.1	5 0	5 0	19.5
5	120	23.8	5 0	5.0	20.0
6	120	26 6	5.5	5 5	20.0
7	120	27 0	7.0	7.0	20.0
8	120	28 8	7.5	7.5	20.0
9	120	30.0	8 3	8.3	20.0
10	120	31 2	8.5	8.5	23.0
11	120	32 0	10.0	10.0	30.0
12	26	34.7	10 0	10 0	30.0
13	0	39.7	10 0	10.0	30.0
14	0	40 0	10.0	10.0	30.0
15	0	42.0	10.0	10.0	30.0
16	0	45 0	10.0	10.0	30.0
17	0	47.5	10 0	10 0	30.0
18	0	50.0	10.0	10.0	30.0
19	0	51.0	10.0	10.0	30.0
20	0	52.6	10 0	10.0	30 0
21	0	54 0	10 0	10.0	25.0
22	0	55.0	7.5	7.5	10 0
23	0	35.5	5.0	5.0	8 0
24	0	44.0	7.0	7 0	10.0
25	0	52 5	7.5	7.5	10.0

injury. It was emaciated, scoured badly, and became partially blind about six weeks before death. An examination of table 1 will also reveal that the calf had a poor appetite during the last thirty days of life.

Cottonseed meal consumption

Since cottonseed meal was fed in sufficient amounts to meet the entire protein requirements for maintenance and lactation, quite

large amounts of the meal were fed daily, especially during heavy milk production. As much as 11 pounds of cottonseed meal were fed per day during the first months of lactation. Food consumption for both lots is shown in table 2. G-3 consumed an average of 7.3 pounds of cottonseed meal per day from the first to second calving time. On the same basis G-1 consumed 7.1 pounds of cottonseed meal; G-5 6.8 pounds; G-7 6 pounds; and G-9 5.9 pounds. Such heavy consumption of cottonseed meal is far beyond the amounts recommended or fed to dairy cattle over a

TABLE 2

Showing feed record of the two lots of animals used in this investigation

	6 MONTHS TO FIRST CALVING							FIRST TO SECOND CALVING						
	Days	C S M.	Average daily	L.O M.	Corn	Silage	Timothy	Days	C S M.	Average daily	L.O M	Corn	Silage	Timothy
Lot I														
G-1	560	1,373	2.45	—	154	7,438	4,554	371	2,629	7 1	—	1,123	9,110	4,438
G-3	541	1,266	2 34	—	220	6,958	4,460	361	2,636	7 3	—	1,141	8,478	4,652
G-5	542	1,259	2 32	—	277	6,002	4,289	407	2,777	6 8	—	1,262	8,859	4,691
G-7	550	1,288	2 34	—	260	6,526	4,185	462	2,768	6 0	—	881	8,820	5,273
G-9	580	1,352	2 35	—	258	7,193	4,756	517	3,060	5 9	—	1,777	9,607	5,874
Lot II														
G-2	564	—		1,682	14	7,416	4,721	362	—		2,563	218	8,843	4,204
G-4	596			1,730	81	7,598	4,845	428			3,790	1,158	8,170	5,254
G-6	551			1,565	48	6,729	4,399	481			3,412	510	9,427	5,717
G-8	547			1,589	100	6,389	4,288	468			4,063	932	10,352	5,649
G-10	614			1,808	50	8,533	5,553							

long period of time. Since the two protein concentrates were fed to meet the entire protein requirments for maintenance and milk production the protein in the ground corn, silage, and timothy hay might be considered as excess protein. The animals in both lots, therefore, consumed about 50 per cent more protein than required by the upper limits of the Morrison Standard.

RESULTS

Health

There has been very little difference in the health of the 2 lots of animals. The heavy feeding of cottonseed meal has not produced

TABLE 3

Showing reproduction record

	Age of first oestrum	First calving					First oestrum period after calving	Second calving					First oestrum period after calving	Third calving		
		Number of services	Blood reaction	Days carried calf	Weight of calf at delivery	Health of calf at delivery		Number of services	Blood reaction	Days carried calf	Weight of calf at delivery	Health of calf at delivery		Number of services	Blood reaction	
Lot I. C.S.M.:																
G-1	357	2	Neg.	275	90	Normal	59	1	Neg.	271	87	Normal*	48	2	Neg.	Pregnant
G-3	430	1	Neg.	272	87	Normal	28	1	Neg.	275	82	Normal	75	5	Neg.	Pregnant
G-5	385	1	Neg.	277	87	Normal	17	2	Neg.	278	95	Normal	128	3	Neg.	Pregnant
G-7	439	1	Neg.	270	63	Normal*	84	1	Neg.	272	72	Normal	132	1	Neg.	Pregnant
G-9	425	1	Neg.	275	75	Normal	124	3	Neg.	284	84	Normal			Neg.	Open
Average	403			274	80.4		62									
Total		6						8								
Lot II. L.O.M.:																
G-2	221	2	Neg.	277	92	Normal	47†	2	Neg.	276	88	Normal*	25	1	Neg.	Pregnant
G-4	407	3	Neg.	276	79	Normal	32	2	Neg.	287	81	Normal	93	1	Neg.	Pregnant
G-6	414	1	Neg.	277	79	Normal	29	3	Neg.	270	79	Normal	87	2	Neg.	Open
G-8	430	1	Neg.	276	80	Normal	79	2	Neg.	240	34	Weak, died				Cow died after previous abortion, uterus inverted
G-10	493	2	Neg.	281	88	Normal	11	4	Pos.	Pregnant						
Average	393			277	85.6		40									
Total		9						12								

* Died 48 hours after birth, due to *B. coli* infection.

† Date of first oestrum period not noted, calculated back 28 days.

symptoms of cottonseed meal injury. G-8 in lot II, which received linseed oil meal, aborted and had an inverted uterus at second calving time. She was negative to the abortion test. It is difficult to determine whether the abortion was due to the ration since none of the other animals receiving the same ration have reacted similarly. After two weeks an infection developed which resulted in death. This infection cannot be attributed to the ration.

TABLE 4

Showing milk and fat production (305 days per lactation)

	FIRST LACTATION		SECOND LACTATION	
	Milk	Fat	Milk	Fat
	pounds	*pounds*	*pounds*	*pounds*
Lot I. C.S.M.:				
G-1.................	9,803	313 6	12,184	376 1
G-3........	10,279	361 4	10,431	348.0
G-5........	10,268	387.8	12,632	369.3
G-7.........	7,613	284 5		
G-9..	10,440	380.4		
Average......	9,861			
Lot II. L.O.M.:				
G-2.................	6,340	235 1	8,744	342.1
G-4................	11,190	413 2	12,786	488 3
G-6	7,932	279.0		
G-8......	9,348	331 3		
G-10................	6,208	189 7		
Average.....................	8,204			

Reproduction

The reproduction records of both lots are summarized in table 3. The birth weights of the calves from lot I compare favorably with those in lot II. G-7's calf in lot I was small, which was probably due to inheritance since the calf's dam was also the smallest animal of either lot.

The loss of 3 calves, 2 in lot I and 1 in lot II, can in no way be attributed to the effects of the ration upon the dams, since the calves were strong and active at birth. All 3 calves died at about

48 hours of age. Apparently a very virulent type of *Bacillus coli* (*Escherichia coli*) was present in the herd at the time these calves were affected. *B. coli* was cultured from the organs and glands of G-2's calf of lot II. Two other calves not on this experiment, born about the same time, died with similar symptoms.

G-8 of lot II which received linseed oil meal aborted her second fetus. The calf weighed only 35 pounds, was very weak, and died in forty-eight hours. The dam was negative to the Bacillus abortus test.

G-1 of Lot I retained her placenta at second calving but passed it normally at third calving.

There was no significant difference between the two lots from the standpoint of reproduction.

Milk production

Milk and butterfat records are shown in table 4. Three animals in lot I receiving cottonseed meal produced more than 10,000 pounds of milk during the first lactation while only one animal in lot II receiving linseed oil meal produced that amount. The animals in lot I which received cottonseed meal produced more milk and butterfat. These differences are probably due to individual variation.

Mastitis

It is a common impression among dairymen that cottonseed meal when fed heavily produces mastitis. In order to determine whether the heavy feeding of cottonseed meal caused an increased number of bacteria in the udder, the bacteria in the milk from each of the animals in both lots was determined over a period of 6 months. Samples were taken for 3 consecutive days each month at the evening milking. The milk of each animal was drawn into a sterile tube in approximately equal amounts from each quarter. One cubic centimeter of milk was then pipetted in 9 cc. of sterile physiological salt solution. One cubic centimeter of this dilution was then plated in duplicate, using nutrient agar. Plates were incubated for 48 hours at room temperature and then incubated for forty-eight hours at 37°C. Results are shown in table 5.

TABLE 5

Showing the number of bacteria per cubic centimeter of freshly drawn milk

DATE	G-1 C.S.M. fed Average daily	G-1 Bacteria per cubic centimeter	G-3 C.S.M. fed Average daily	G-3 Bacteria per cubic centimeter	G-5 C.S.M. fed Average daily	G-5 Bacteria per cubic centimeter	G-7 C.S.M. fed Average daily	G-7 Bacteria per cubic centimeter	G-9 C.S.M. fed Average daily	G-9 Bacteria per cubic centimeter	G-2 L.O.M. fed Average daily	G-2 Bacteria per cubic centimeter	G-4 L.O.M. fed Average daily	G-4 Bacteria per cubic centimeter	G-6 L.O.M. fed Average daily	G-6 Bacteria per cubic centimeter	G-8 L.O.M. fed Average daily	G-8 Bacteria per cubic centimeter	G-10 L.O.M. fed Average daily	G-10 Bacteria per cubic centimeter
1928																				
October 17	4.5	Dry	6.4	400	7.2	80	6.9	30	3.0	15	3.0	Dry	9.4	295	8.4	25	9.9	25	3.0	Not calved
October 18		Dry		1,660		65		40		100		Dry		885		65		60		Not calved
October 19		Dry		3,320		260		30		60		Dry		980		65		70		Not calved
November 14	2.2	Dry	2.0	Dry	7.0	65	6.0	50	6.0	25	2.8	Dry	8.8	265	7.2	75	9.4	40	3.0	Not calved
November 15		Dry		Dry		430		15		10		Dry		200		30		15		Not calved
November 16		Dry		Dry		155		5		120		Dry		190		10		45		Not calved
1929																				
December 18	7.5	15	2.0	Dry	5.8	130	6.4	10	9.9	140	10.5	315	8.0	305	7.0	15	9.4	75	4.5	65
December 19		10		Dry		160		40		225		1,610		130		65		235		140
December 20		15		Dry		125		10		185		525		50		20		35		65
January 18	10.8	20	11.7	970	2.0	Dry	6.2	170	10.2	285	10.5	205	9.6	20	6.8	20	8.4	40	11.4	60
January 19		40		120		Dry		20		195		270		55		130		70		110
February 25	10.2	30	11.8	960	2.0	Dry	5.6	15	9.9	25	10.6	5	2.6	Dry	6.8	5	8.8	15	9.0	15
February 26		30		4,920		Dry		10		60		0		Dry		15		5		10
February 27		20		2,890		Dry		5		20		5		Dry		15		10		10
March 19	9.9	60	10.5	2,870	6.0	25	5.3	45	6.6	70	10.5	290	2.6	Dry	6.4	85	8.2	30	9.3	25
March 20		40		2,250		25		40		35		125		Dry		20		0		10
March 21		20		400		15		165		20		60		Dry		15		5		5

Apparently all bacterial counts were within a normal range with the exception of one animal, G-3 in lot I, which had a rather high count.   An examination of the milk from the separate quarters revealed a high count in one quarter.   However, this cow has never shown any evidence of mastitis.   The animals in both lots have been free from this disease although other animals in the experimental herd have had mastitis.   Milking machines were used in milking the animals.

DISCUSSION OF RESULTS

The results of this investigation lend further support to the theory set forth in a previous paper (1) that cottonseed meal injury in dairy cattle is due to a dietary deficiency caused by lack of a factor or factors carried by good quality hay.   In the previous paper (1) symptoms of cottonseed meal injury were produced in two calves which did not receive cottonseed meal in the ration. The North Carolina Station (8) reported death of animals fed linseed oil meal, peanut meal, and soybean meal, as well as with cottonseed meal where a poor quality of roughage was used.   The cottonseed meal fed in this investigation produced symptoms of cottonseed meal injury in calf C-80 where a poor quality of roughage was fed.   However, when a good quality of roughage was fed, large amounts of cottonseed meal were consumed by the animals in this experiment without injury.

The results fail to support a commonly accepted idea that the heavy feeding of cottonseed meal to dairy cattle results in so called injury due to the presence of a poisonous principle, gossypol. The cottonseed meal used in this investigation was purchased on the open market.   As much as 11 pounds of cottonseed meal were fed per day during the first few months of lactation without injury.   G-1 consumed 7.1 pounds; G-3 7.3 pounds; G-5 6.8 pounds; and G-7 6 pounds of cottonseed meal on the average per day from first to second calving, which includes a two months dry period. The health of these animals have been good thus far.

Possibly gossypol is either non-toxic to the bovine or in the process of extraction of the oil from the raw cottonseed sufficient of the "free" gossypol is converted to the "bound" gossypol to

render the meal sufficiently non-toxic for use by dairy cattle. This problem is under investigation at the present time at this Station. In connection with toxicity of cottonseed meal for different species Gray and Ridgeway (10) noted that the cottonseed meal which they fed ewes with no ill effects caused the death of several hogs in the swine experimental work.

Milk production has not been interferred with by heavy consumption of cottonseed meal. G-1 produced 9,803 pounds; G-3 10,279 pounds; G-5 10,268 pounds; G-7 7,613 pounds; and G-9 10,440 pounds of milk during the first 305 days of the first lactation period.

The heavy feeding of cottonseed meal to dairy heifers from 90 days to approximately four years of age resulted in normal reproduction when compared with the check group which received linseed oil meal along with timothy hay, corn silage, and yellow corn.

There was no significant difference between the 2 lots in strength of calves at birth. Two calves from lot I fed cottonseed meal and one calf from lot II fed linseed oil meal died at 48 hours of age, although they appeared normal and healthy at birth. The cause of death was thought to be due to the presence of a virulent *B. coli* infection in the herd, since 2 other calves born about the same time and not on this experiment died with similar symptoms. *B. coli* was cultivated from the organs of G-2's calf whose dam received linseed oil meal. Several calves not on this experiment have died with similar symptoms since then. *B. coli* was cultivated also from these calves.

G-8 which received linseed meal aborted her second fœtus with no apparent cause. This is the only calf in either group which was not strong at birth. There was but little difference between the birth weights of the calves of the two lots.

Although the animals used in this investigation were kept among animals positive to *B. abortus* test, all of the animals in both lots have remained free from the disease except G-10 receiving linseed oil meal, which became positive but has not aborted. The heavy feeding of cottonseed meal has not made these animals more susceptible to *B. abortus* infection. The results indicate that the liberal feeding of cottonseed meal does not affect repro-

duction adversely when fed with ample hay of high quality and corn silage.

The heavy feeding of cottonseed meal did not increase the susceptibility of heavy milking cows to udder infection. In previous work the normal number of bacteria in the udder of cattle was determined by Copeland and Olson (15) who found that milk drawn from each quarter of the udder of 40 cows gave an average count of 1,541 per cc. of milk. Extreme bacterial counts ranged from 0 to 347,000. Harding and Wilson (16) examined 1,230 samples of milk from the udders of 78 normal cows. They found an average of 428 bacteria per cc. of milk.

In this investigation all the bacterial counts were within normal range except for G-3 in lot I which received cottonseed meal. However, no evidence of mastitis was observed in this animal. These results were secured even though the animals used in this investigation were kept with other cows which had mastitis. Our results are not in accord with those secured by Moore (2) who reported that the heavy feeding of cottonseed meal caused mastitis and loss of quarters, whereas little trouble occurred in a lot which did not receive cottonseed meal although all lots produced similarly. Moore did not state whether or not both lots were equally exposed to infection.

SUMMARY

The results of this investigation lend further support to the theory that cottonseed meal injury in dairy cattle is due to the lack of a factor or factors carried by good quality hay.

Liberal feeding of cottonseed meal to dairy cattle from three months to four years of age along with ample hay of high quality and corn silage had no apparent effect on health, reproduction or lactation.

Liberal feeding of cottonseed meal to lactating cows did not increase susceptibility to udder infection.

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PLATE 1

SHOWING G-3 OF LOT I AT 4 YEARS OF AGE JUST PREVIOUS TO 3RD CALVING

This animal consumed an average of 7.3 pounds of cottonseed meal daily from first to second calving.

PLATE 2

SHOWING STRENGTH OF G-9's CALF OF LOT I AT BIRTH

This calf died at forty-eight hours of age due to B. coli infection.
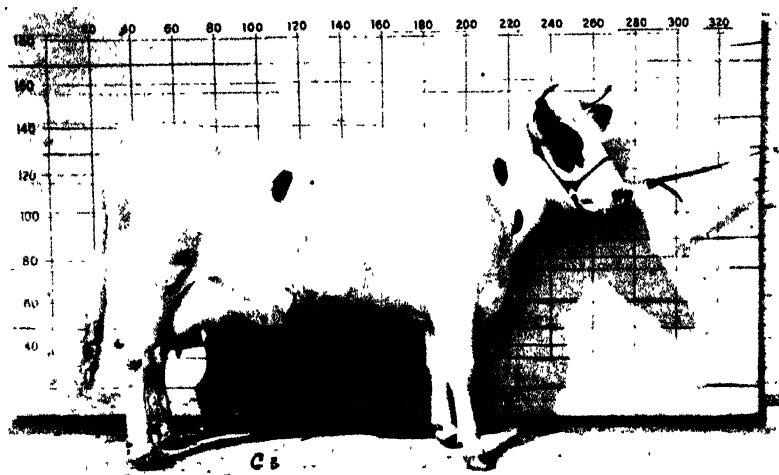
PLATE 1
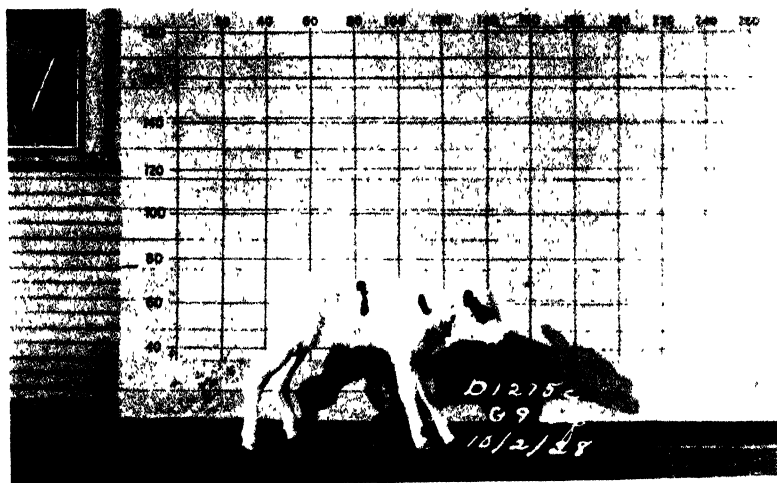
PLATE 2

FAT SOLUBLE VITAMINS

XXXI. BUTTER FAT: ITS ANTIRACHITIC PROPERTIES AND ITS ARTIFICIAL ACTIVATION*

H. STEENBOCK AND ALICE M. WIRICK

WITH THE COÖPERATION OF BLANCHE M. RIISING

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For many years the inference might have been drawn from general premises that butter fat is low in antirachitic potency. If butter fat were not poor in this dietary essential the incidence of rickets in children fed on cows' milk should be a very rare occurrence, but as a matter of fact it is well known that the vast majority of infants fed cows' milk develop rickets during the winter months.

Some years ago, however, Mellanby (1) in his pioneering experiments was very much impressed with the power of butter fat in protecting puppies against deficient calcification of bone. But he found that cod liver oil was far superior. McCollum, Simmonds, and Becker, and Shipley (2) found that while butter fat contained the calcium depositing factor, it was present in much smaller amounts than in cod liver oil and in other fish oils which they examined. McCollum, Simmonds, Shipley, and Park (3) found that 3 per cent of cod liver oil prevented the occurrence of rickets while even 20 per cent of butter fat failed to induce normal bone growth. Jones, Steenbock, and Nelson (4), seeking to duplicate the experimental work of others, which was designed to point out the disproportionate activity of butter fat and cod liver oil in their ability to cure ophthalmia as contrasted with rickets, came to the conclusion that cod liver oil might in certain instances be 200 times as active as butter fat in inducing normal calcification of bone.

In 1924 experiments from this laboratory (5–10) as well as those from the laboratory of Hess (11–16) showed definitely that

the antirachitic potency of numerous fats could be increased by exposure to ultra violet radiations such as those produced by a quartz mercury vapor lamp.

Steenbock and Black (17) showed that butter fat could be made decidedly antirachitically active by the same procedure. They also showed that oils such as cocoanut oil, corn oil, oleo oil, peanut oil and cottonseed oil, which had become rancid, could not be made potent by such exposure. This was taken as evidence that the induction of antirachitic properties in our ordinary fats was not due to the action of the ultra violet irradiations upon the fats themselves, but upon certain substances of a rather labile character contained in solution in the fats and oils. Steenbock, Black, and Nelson (8) had furthermore previously reported that apparently all the antirachitic potency found in irradiated fats was localized in the unsaponifiable constituents contained therein. In the same year (9) they obtained evidence that while sterols of ordinary purity and even after numerous recrystallizations could become antirachitically activitated, in view of the fact that sterols are separated from one another with such great difficulty, the evidence that all activation was confined to the action of the ultra violet rays upon cholesterol, for example, was not at all convincing (17). Therefore it was generally referred to the activation of unsaponifiable lipoïds as a group (6). That sterols were apparently activated was also reported by Hess and Weinstock (15). Hess, Weinstock, and Helman (16) furthermore separated an active unsaponifiable constituent from irradiated linseed oil.

While it thus became generally known that antirachitic activation was effected not by the action of the ultra violet rays upon proteins, carbohydrates and fats, but upon certain unsaponifiable constituents, presumably sterols, it remained for Windaus and Hess (18), Rosenheim and Webster (19, 20, 21), and Heilbron, Kamm, and Morton (22) to discover that the activation was resident in a sterol known as ergosterol, which apparently occurs widely distributed in small amounts, being concentrated in the unsaponifiable fraction and frequently crystallized out with other plant and animal sterols. While formerly, positive antirachitic tests were obtained when a milligram of ordinary choles-

terol was fed to rachitic animals, with irradiated ergosterol it was found possible to obtain results with 0.0001 mgm. or even less as a single dose.

It is still an open question (23–26) whether other substances besides ergosterol can be made active by ultra violet irradiation. Part of the difficulty in determining this lies in the fact that chemical tests for sterols in general are infinitely less delicate than the biological tests, and furthermore, inasmuch as it has been shown that derivatives of sterols or sterol mixtures can be activated (9, 20) it must be recognized that highly purified activatable starches and proteins may owe their activatability to their existence in combination with ergosterol. But to the present time it has not been found possible in this laboratory to prepare certain non-activatable starches, for example, by long continued extraction with ether and alcohol which should extract free ergosterol.

In the experiments discussed in this paper, presentation of results and experience in the use of the irradiation process is limited to butter fat. Here the problem simplified itself materially because in the irradiation of butter fat we are not concerned with its effect upon other substances, such as enzymes, proteins or the water soluble vitamins which might be injured. Primarily the problem resolved itself into defining a treatment effective in the induction of antirachitic activity without concomitant destruction of vitamin A, palatability, or color. It was soon determined that the process of irradiation need not be carried out for such a length of time or with such intensity as to destroy palatability or to cause bleaching, because the maximum antirachitic potency can be induced in far shorter time than that which will cause the aforementioned undesirable changes. The most difficult conditions which had to be met were those which concerned themselves with the prevention of vitamin A destruction. We found it necessary to carry out a rather extended series of experiments to define the conditions accurately.

EXPERIMENTAL

Our first experiments concerned themselves with the definition of the conditions necessary to secure maximum activation of

butter fat. We reserved for our experiments fifty pounds of June butter fat manufactured in the University Creamery from the milk of cows on pasture. This was kept in cold storage and small samples were melted rapidly in an oven at approximately 95°C., filtered through paper and then directly irradiated as required. For the irradiation there was used for the most part an Alpine Sun Lamp kindly loaned us by the Hanovia Chemical and Manufacturing Company. In some of our experiments, however, we also used a Cooper-Hewitt mercury vapor lamp of the B.Y. type. The distance of exposure was kept uniform at 18 inches. The butter fat was kept melted during the exposure, usually being kept at a temperature of approximately 35°. In no case was the temperature allowed to exceed 50° because of possible harmful effects. The exposure was always carried out in pie tins $7\frac{3}{4}$ inches in diameter. The pie tins were kept bright in order that the rays penetrating the butter fat completely might reflect their action from the bottom of the container. Though not proven, it was believed that this might be a very important factor because for the most part our experiments were limited to the exposure of a layer of butter fat not less than 0.08 cm. in thickness and not more than 1.28. It was not found practical to use thinner films or layers because of the difficulty of maintaining uniform distribution of the fat on the bottom of the containers. Outside of the variations in depth of material, we also introduced a time variable. This ranged from 10 minutes to 16 hours.

The antirachitic potency of the treated butter fat was determined with the technique developed in this laboratory in the course of the last few years. All the animals, namely rats, used for the experiments were produced in the laboratory on our modified stock ration consisting of yellow corn 71, linseed oil meal 15, casein 5, alfalfa meal 2, bone ash 1, sodium chloride 1, butter fat 5, fed with fresh milk and water ad libitum. Litters produced by animals on this diet were reduced to 6 in number when they were a week old, and were used for the experiment only after they had attained a weight of approximately 60 grams in not more than 4 weeks from the time of birth.

Two types of experiments were used for the testing of the irra-

diated product. The one given first consideration was based upon the Johns Hopkins technique (27) in which the diagnosis of the curative properties of a treated product is dependent upon the deposition of calcium in the metaphyses of animals in which rickets had been produced by feeding our 2965 ration (17). In this technique the animals are fed the aforementioned ration consisting of yellow corn 76, wheat gluten 20, calcium carbonate 3, sodium chloride 1, for a period of not less than 3 weeks. Sometimes at 3 weeks, though more often at about 24 to 25 days, young rats have developed a severe rachitic condition indicated not only by enlargement of joints such as the wrists, but also by a very shambling, so-called rachitic gait. When animals with this degree of rachitic involvement have introduced into their 2965 ration an antirachitically active agent such as cod liver oil, irradiated cereals, or fats in sufficient amounts, calcium deposition in the form of a calcium phosphate complex occurs in the rachitic lesions, this being especially discernible in the metaphyses of the long bones.

In the technique as used in our laboratory, we keep the animals for a period of 10 days after the introduction of the irradiated substance into the diet before we make a diagnosis. Usually we have made it a practice to feed all of the addition in the first 50 grams of ration fed in this period. This secures complete consumption of the antirachitic addition because animals usually consume in the neighborhood of 7 grams daily. Due precautions are taken that the observed results will not be complicated by a starvation factor, because it is well known that starvation even for a few days will likewise produce a healing of the lesions. For this purpose consumption records of the animals are kept daily. At the end of the 10-day period the animals are killed with ether, and the wrists, after dissecting away skin and muscles, are placed in 10 per cent formaldehyde for a period varying from 4 hours to as many days. They should not be kept for many weeks because this has frequently been found to result in the resolution of the calcium deposits. Short of this, at any time after 4 hours, the radii and ulnae ends are split with a sharp scalpel, then they are immersed in a 1.5 per cent solution of silver nitrate and exposed to a Mazda

light. With deposition of silver in the area of the bone tissue impregnated with lime salts, the bones are removed to water and kept there for observation. The extent of the calcium deposition in the rachitic metaphyses as revealed by this technique is used as a measure of the antirachitic potency of the material tested.

In these experiments we used butter fat which had been irradiated at 0.08, 0.16, 0.32, 0.64, and 1.28 cm. in depth for periods of 30 minutes and 2 hours. It was fed at levels of intake of 20, 40, 80 and 120 mgm. daily, separately and continuously for 10 days instead of being incorporated in the ration. We had frequently observed decided variations in the results obtained by the Johns Hopkins method (27) and it was for that reason that we projected our experiments on such an extensive scale. For the entire series we used a total of 43 litters of rats, all of which had been produced with our standard technique in the laboratory, and all of which were kept under as near identical conditions as we found it possible to define them.

The data accumulated as a result of this work are presented in part in table 1 for the reader's own deductions. They were selected as a group without elimination of any of the experiments of the series, so that they can be taken as representative of the results obtained as a whole. As each litter of rats contained only 6 individuals, it was, of course, experimentally impossible to make all comparisons of the different depths of exposure as well as time intervals within each litter. The table therefore shows in its upper part, litter comparison over 30 minutes' exposure for all depths, with 2 hours' exposure with a depth of 0.32 cm. The lower part of the table shows comparisons within litters of an exposure for 30 minutes at 0.32 cm. depth, with 2 hours' exposure at all depths.

Results in the table are expressed in terms of "plus" signs; one "plus" sign is used for a narrow line of calcium deposits in the rachitic metaphyses, two for a medium line, three for a wide line, four, approximately complete healing, and five, complete healing. The data indicate in a general way that with 2 hours' exposure, the activation secured was independent of depth of butter fat exposed. With 30 minutes' exposure a pronounced

decrease in activation occurred at the greater depths, namely, 0.64 and 1.28 cm. The disconcerting revelation in all of our curative experiments was the fact that when small amounts of butter fat produced definite healing, increased intake did not result in a commensurate increase in calcium deposition. This was apparently most evident when the potency approached that necessary to produce complete healing. When mere incidence of calcium deposits was used as a criterion, the results with successive increment of dose were apparently more consistent.

From our data we are led to caution investigators who seek to use the Hopkins method (27) for quantitative purposes. In all cases, a large number of animals should be used on each level of intake; certainly the number should not be less than 6, and preferably greater. And, irrespective of the number used, the incidence of a definite continuous calcium line at a certain level of intake should be taken as the criterion in preference to successive increases in the degree of healing.

For the main program of our investigation we made use of a prophylactic type of experiment. In this technique the antirachitic supplement is added to our 2965 ration as soon as the animals are put on the experiment, which, as already stated, is done when they attain a weight of 60 grams. When very small amount of a comparatively tasteless supplement are used, so small that they cannot have an effect upon the palatability and therefore the consumption of the ration, no attempt to equalize the consumption of the ration between the various groups is made. If this is not the case, then each group of animals is given the same amount of the supplemented ration day by day, the consumption of all being determined by the lowest amount consumed by any animals in the series. This general procedure of equalized consumption is departed from only when the animal consuming the least is obviously abnormal. Usually 4 animals are put on each level of supplement and of these 4 animals each individual is from a different litter. Furthermore, every litter used in the experiments is given a representative in the group. In this manner it is believed that individual variations in litter are equalized as much as possible. At the end of 5 weeks' feeding,

TABLE 1

Antirachitic activation of butter fat as determined by cure of rickets in rats

AMOUNT FED DAILY	BUTTER FAT IRRADIATED 30 MINUTES AT DEPTHS OF					BUTTER FAT IRRADIATED 2 HOURS AT DEPTHS OF					LITTER NUMBER
mgm.	0.08 cm.	0.16 cm.	0.32 cm.	0.64 cm.	1.28 cm.	0.08 cm.	0.16 cm.	0.32 cm.	0.64 cm.	1.28 cm.	
20	+ + +	+ +	? ++ +	− −	− + −			+ ++ +			2 15 27
40	+ + + + +	+++ +++ +++ +	+ ++ ++ +	− +++ ·+ − +	+ + ? + +			+++ +++ + +			3 16 31 35 39
80	+ ++ + ++ + +	+++ +++ ++	++ +++ +	++	++ ++ ++			++ ++ ++			6 20 32
120	+ ++ ++ +	·+ ++	++ +++ +	++ ++	++ ++			++ +			7 22
20			− +			+ ++ +	+ ++	+ +	+++ ++	++ ++	9 24
40			+++ +++			+ ++ +	++ ++ +	++ ++ +	+++ +++ +	+ + ++	10 23 36

80			+ ++ ++			- +++ ++ +	- ++ +++ +	+++ +++ ++	++++ ++++ ++++	+++ +++ +++ +	40 12 25
120			++ +++ ++			- ++ +++ +++	- +++	+++ +++ ++	+++ +++ ++	++ +++	13 26

? = reaction questionable; + = narrow line of calcium; ++ = medium line of calcium or medium metaphyses; +++ = wide line of calcium or narrow metaphyses; ++++ = approximately complete healing; +++++ = complete healing.

the animals are killed with ether, the femora and humeri are dissected out, carefully cleaned free from adhering muscle and connective tissue, and then extracted with alcohol without drying, from 4 to 7 days.   The extraction is carried out in a Soxhlet extractor the alcohol being changed at least once a day in order to remove water extracted from the bones.   After this thorough extraction, the bones are dried at 100° and then ashed in an electric muffle furnace.   Comparison of the antirachitic potency is based upon the percentage of ash in the bones.   For control purpose, when using this technique we also frequently removed the wrists as discussed in the previously described technique, and very frequently we also examined the costo-chondral junctions for rachitic enlargement.

This method has been previously used, but the first results obtained therewith in the present experiments are shown in table 2. This table brings out the comparative induction of antirachitic potency in butter fat when irradiated with the Alpine Sun Lamp for 10 minutes as compared with 30 minutes.   The irradiation in this and all subsequent experiments, unless otherwise indicated, was carried out with the butter fat at a depth of 0.16 cm.   This depth was chosen provisionally because of the fact that with it, it was easy to maintain continuity of film and furthermore, the curative experiments had shown that at this depth satisfactory activation was secured with an exposure of 30 minutes.   In the experiments of table 2 the butter fat was fed at a level of 10 and 20 mgm. daily intake in separate portions given to the rats daily in addition to ration 2965 fed ad libitum.   Records of the consumption of ration 2965 were not kept because it was presumed that consumption would be uniform.   As stated previously, each group of animals had a representative from each one of the litters used.   Therefore, in the table the values in the same vertical column represent the analysis of rats from the same litter.   In these experiments, both femora were analyzed in order to determine the variations in femora taken from the same animal.   It will be noticed that individual analysis of the different femora taken from the same rat vary from 0.06 to 1.34 per cent.   Taking the averages of each group as a whole, it is seen that 20 mgm. of

TABLE 2

Antirachitic activation of butter fat irradiated at a depth of 0.16 cm. for ten minutes as compared with thirty minutes

RAT NUMBERS	BUTTER FAT ADDITION TO RATION 2965		PER CENT ASH				GRAND AVERAGE	WIDTH OF METAPHYSES	SIZE OF COSTO-CHONDRAL JUNCTIONS
233–236	10 mgm. daily; irradiated 10 minutes	Femurs	39.0	37.81	44.20	44.31		Narrow metaphysis to almost normal epiphyseal line	Pronounced to medium enlargement
			40.07	38.82	44.57	44.01			
		Average	39.58	38.32	44.39	44.16	41 61		
237–240	20 mgm. daily; irradiated 10 minutes	Femurs	46.08	45 30	45.00	42 84		Almost normal epiphyseal line	Normal (slight enlargement in rat 238)
			46.20	44.25	43.74	43.56			
		Average	46.14	44.78	44.37	43.20	44.62		
240–252	10 mgm. daily; irradiated 30 minutes	Femurs	40.40	45.69	39.15	45.57		Narrow metaphysis	Medium to slight enlargement
			40.02	44.82	39.21	46.41			
		Average	40.21	45.26	39.18	45.99	42.66		
253–256	20 mgm. daily; irradiated 30 minutes	Femurs	45.59	49.36	49.28	49.35		Almost normal	Normal
			45.35	48.02	48.54	48.30			
		Average	45.47	48.69	48.91	48.83	47.97		

TABLE 3

Antirachitic activation of butter fat irradiated at a depth of 0.16 cm. for thirty minutes as compared with 180 minutes

RAT NUMBERS	BUTTER FAT ADDITION TO RATION 2965		PER CENT ASH	AVERAGE	WIDTH OF METAPHYSES	SIZE OF COSTO-CHONDRAL JUNCTIONS
137–140	5 mgm. daily; irradiated 30 minutes	Femurs	38.05 32.55 36.36 37.18	36.04	Trace of calcification	Pronounced enlargement
141–144	20 mgm. daily; irradiated 30 minutes	Femurs	50.11 49.12 49.42	49.19	Normal epiphyseal line	Normal
145–148	40 mgm. daily; irradiated 30 minutes	Femurs	52.76 47.66 45.94 48.01	48.59	Normal epiphyseal line	Normal
149–152	5 mgm. daily; irradiated 2 hours	Femurs	34.75 37.61 38.78	37.05	Medium metaphysis	Pronounced enlargement
153–156	20 mgm. daily; irradiated 2 hours	Femurs	49.14 49.29 44.50 49.02	47.99	Normal epiphyseal line	Normal
157–160	40 mgm. daily; irradiated 2 hours	Femurs	50.27 46.20 47.61 46.68	47.69	Normal epiphyseal line	Normal

irradiated butter fat fed daily produces more calcification than 10 mgm., and furthermore, that a 30-minute exposure of the aforementioned sample produced from 1 to 3 per cent more calcification than the 10 minute irradiation.

In table 3 are presented data obtained in the same manner as those presented in table 2 with the exception that the levels of intake were 5, 20 and 40 mgm. daily. Ordinary butter fat was irradiated for 30 minutes as contrasted with 120 minutes, the primary object of this series being to determine whether or not 30 minutes' exposure effected complete activation. Inasmuch as the femora had been found to give fairly consistent data as far as calcification was concerned in these experiments, only one femur was analyzed. Gross inspection of the table reveals immediately that 30 minutes' exposure was as efficient in inducing complete activation as 2 hours' and that with the exposure of 30 minutes, 20 mgm. of butter fat daily was sufficient to produce complete calcification.

In table 4 are presented data comparing the Alpine Sun Lamp, manufactured by the Hanovia Chemical and Manufacturing Company, with the Cooper Hewitt lamp of the B.Y. type. Both lamps when used were practically new, the former having been used for 185 hours at low intensity, while the Cooper Hewitt had been used for 82 hours. Both burners were free from blemishes and were in excellent condition. The former was run from our A.C. lighting circuit on 110 volts, as specified by the maker; the latter was run on D.C. current with a voltage of 50 and a current density of 4 amperes. The butter fat in each case was exposed under the same conditions as to temperature, container, and original source. Litter distributions among the different groups were the same as employed in previous experiments and analyses were limited to both femora.

It is seen from table 4 that while 10 minutes' exposure with the Alpine Sun Lamp gave slightly better results than 10 minutes' exposure with the Cooper Hewitt lamp, 20 minutes' exposure with the Alpine Sun Lamp gave slightly lower results than 20 minutes' exposure with the Cooper Hewitt lamp. This shows that the differences in values observed were due to experimental error or

TABLE 4

*Comparative activatability of butter fat irradiated by the Alpine Sun Lamp and by the Cooper-Hewitt Lamp**

RAT NUMBERS	BUTTER FAT ADDITIONS TO RATION 2965		PER CENT ASH IN FEMURS						GRAND AVERAGE	WIDTH OF METAPHYSES	SIZE OF COSTO-CHONDRAL JUNCTIONS
301–306	20 mgm. irradiated 10 minutes by Alpine Sun Lamp	Femurs	37.00	41.17	41.20	44.25	46.18	46.18	39.33		Medium metaphysis	Slight enlargement
			37.35	41.67	41.25	45.41	46.17		39.72			
		Average	37.18	41.42	41.23	44.83	46.18	39.53	41.73		
307–312	20 mgm. irradiated 20 minutes by Alpine Sun Lamp	Femurs	45.46	46.22	44.95	48.83	40.72	42.94			Very narrow metaphysis	Almost normal
			45.35	46.62	45.20	48.52	40.24	42.96				
		Average	45.41	46.42	45.08	48.68	40.48	42.95	44.83		
313–318	20 mgm. irradiated 30 minutes by Alpine Sun Lamp	Femurs	44.81	41.69	47.78	47.95	45.36	46.22			Very narrow metaphysis	Almost normal
			43.87	41.82	48.18	46.89	45.56	47.54				
		Average	44.34	41.76	47.98	47.42	45.46	46.88	45.64		
319–324	20 mgm. irradiated 5 minutes by Cooper-Hewitt lamp	Femurs	33.21	37.91	41.67	38.06	39.10	37.43			Medium metaphysis with cupping	Pronounced enlargement
			33.73	38.12	42.88	37.35	38.85	37.36				
		Average	33.47	38.02	42.28	37.71	38.98	37.40	37.97		
325–330	20 mgm. irradiated 10 minutes by Cooper-Hewitt lamp	Femurs	43.37	32.77	45.65	40.00	41.98	39.93			Medium metaphysis	Medium enlargement
			43.08	32.70	46.14	39.62	41.75	39.82				
		Average	43.23	32.74	45.90	39.81	41.87	39.88	40.57		
331–335	20 mgm. irradiated 20 minutes by Cooper-Hewitt lamp	Femurs	49.31	50.23	48.71	42.99	43.46				Very narrow metaphysis	Normal
			48.19	50.52	48.67	43.17	43.86					
		Average	48.75	50.38	48.69	43.08	43.66		46.91		

* Alpine Sun Lamp had been run for 185 hours (low). Cooper-Hewitt Lamp had been run for 82⅓ hours.

inherent variation in the calcium content of animals, from the same litters, irrespective of the antirachitic potency of the materials fed. We therefore feel safe in concluding that the quartz mercury vapor lamps of the two types compared are essentially equally proficient in effecting activation.

We have already discussed the paucity of butter fat in vitamin D content. We were therefore interested in determining the comparative potency of untreated butter fat with butter fat which had been irradiated for 30 minutes at a depth of 0.16 cm., which represents the most favorable conditions as determined by the aforementioned experiments. The results of experiments to this end are presented in table 5. Taking the usual precautions in regard to litter distribution of animals, and so forth, untreated butter fat was fed daily in individual portions contained in small dishes, separate from ration 2965 which formed the bulk of the diet of the animals. Of the untreated butter fat there were fed 80, 160, and 400 mgm. daily; of the irradiated butter fat 20, 10, and 5 mgm. The 5- and 10-mgm. portions were measured out in ether solution on small portions of ration 2965, in order to secure complete consumption. Included in the table are percentage values for butter fat which had been calculated on a basal intake assuming that every rat consumed approximately 8 grams of the ration daily. These percentage values, however, are to be taken as an approximation only. The table shows that 20 mgm. of irradiated butter fat were equal in antirachitic potency to 400 mgm. of the untreated. This represents a multiple of 20 times the activity of ordinary June butter fat as compared with the treated production.

It has already been brought out in our introduction that the excessive treatment of fats, such as olive oil and cod liver oil, under ultra violet rays brings out a total destruction of antirachitic potency. That this relation also obtains in the case of butter fat is presented in table 6. As we were not so much concerned with the quantitativeness of the reaction as with the demonstration of its occurrence, we felt that data satisfactory for our purpose could be obtained by the use of the less laborious, though more inaccurate curative experiments as contrasted with the

TABLE 5

The comparative amounts of vitamin D in butter fat natural and irradiated for 30 minutes at a depth of 0.18 cm.

RAT NUMBERS	BUTTER FAT ADDITION TO RATION 2965		PER CENT ASH IN FEMURS				GRAND AVERAGE	WIDTH OF METAPHYSES	SIZE OF COSTO-CHONDRAL JUNCTIONS
277–280	1 per cent or 80 mgm. untreated	Femurs	32.38	32.94	27.52	28.25		Wide rachitic metaphysis	Pronounced enlargement. Extreme angulation
			31.72	32.98	27.98	30.11			
		Average	32.05	32.96	27.75	29.18	30.49		
281–284	2 per cent or 160 mgm. untreated	Femurs	39.07	42.29	33.93	31.65		Wide rachitic metaphysis to medium metaphysis	Pronounced to medium enlargement
			38.78	42.25	34.18	31.42			
		Average	38.93	42.27	34.07	31.54	36.70		
285–288	5 per cent or 400 mgm. untreated	Femurs	44.76	48.52	46.70	42.16		Narrow metaphysis	Almost normal
			44.86	48.89	46.49	43.23			
		Average	44.81	48.71	46.60	42.70	45.70		
289–292	0.25 per cent or 20 mgm. irradiated 30 minutes	Femurs	47.49	48.08	43.16	45.27		Very narrow metaphysis	Normal
			46.34	47.73	42.75	45.05			
		Average	46.92	47.91	42.96	45.16	45.73		
293–296	0.12 per cent or 10 mgm. irradiated 30 minutes	Femurs	39.71	47.75	37.51	43.72		Narrow metaphysis	Slight enlargement
			39.74	47.09	36.31	43.31			
		Average	39.73	47.42	36.91	43.52	41.89		
297–300	0.06 per cent or 5 mgm. irradiated 30 minutes	Femurs	26.67	44.26	32.60	35.15		Medium metaphysis	Medium enlargement
			26.11	44.63	32.59	35.57			
		Average	26.39	44.45	32.60	35.36	34.70		

prophylactic determinations based on ash content. Butter fat for these experiments was irradiated at a depth of 0.16 cm. for 30 minutes, 5 hours, 10 hours, and 16 hours. For the short exposures

TABLE 6

The effect of excessive irradiation on the antirachitic activity of butter fat

LITTER NUMBER	RAT NUMBER	BUTTER FAT ADDITIONS TO RATION 2965			INITIAL WEIGHT	FINAL WEIGHT	AVERAGE GRAMS RATIONS 2965 CONSUMED DAILY	LINE TEST
			per cent	mgm.				
	8268	Irradiated 30 minutes at 35°C.	0.5	31.0	95	90	6.2	++
	8269	Irradiated 10 hours at 35°C.	0.5	49.0	97	112	9.8	−
49	8270	Irradiated 16 hours at 50°C.	0.5	38 0	96	97	7.6	−
	8271	Irradiated 30 minutes at 35° C. then heated for 15.5 hours at 50°C.	0.5	43.0	86	93	8.6	++ to +++
	8272	Irradiated 30 minutes at 35°C.	0.5	35.5	86	88	7.1	+++
50	8273	Irradiated 5 hours at 35°C.	0.5	40.0	75	80	8.0	+++
	8274	Irradiated 10 hours at 35°C.	0.5	42.5	85	92	8.5	++
	8275	Irradiated 16 hours at 50°C.	0.5	42.0	82	88	8.4	−
	8276	Irradiated 30 minutes at 35°C.	0.5	28.0	85	80	5.6	+++
	8277	Irradiated 5 hours at 35°C.	0.5	29.0	70	68	5.8	+++++
51	8278	Irradiated 10 hours at 35°C.	0.5	43.0	86	98	8.6	++
	8279	Irradiated 16 hours at 50°C.	0.5	43.5	90	98	8.7	−
	8280	Irradiated 30 minutes at 35°C. then heated for 15.5 hours at 50°C.	0.5	25.5	83	79	5.1	++++
	8281	Irradiated 30 minutes at 35°C.	0.5	31.5	96	97	6.3	++
	8282	Irradiated 16 hours at 50°C.	2.0	118.0	98	97	5.9	−
52	8283	Irradiated 16 hours at 50°C.	2.0	158.0	98	102	7.9	−
	8284	Irradiated 30 minutes at 35°C. then heated for 15.5 hours at 50°C.	2.0	148.0	92	89	8.4	++++

it was found satisfactory to keep the butter fat melted at 35°, but in the exposure of 16 hours the temperature inadvertently went up to 50°. To determine if the higher temperature in itself was a factor in the destruction of the antirachitic potency induced, we

irradiated one sample for 30 minutes at 35° and then followed this treatment with heating at 50° for 15½ hours, to make a total of 16 hours. The butter fat was incorporated in ration 2965 at levels of 0.5 to 2 per cent and these rations were fed throughout the 10-day period. Of the total intake of butter fat calculated from the total consumption of ration, the results as shown in table 6 indicated that 10 hours' exposure caused pronounced destruction, and 16 hours caused practically complete destruction as far as the low level of butter fat intake indicated. Inasmuch as activated butter fat heated for the same period of time proved to be potent, the destruction observed was not attributable to heat itself.

With the conditions for maximum activation of butter fat established, it was of interest to us to determine if this activity was a stable property under storage conditions. To obtain data on this, irradiated butter fat was stored in quart Mason jars at room temperature and in the refrigerator in the absence of light. The experiment was begun in July and terminated the following March. The room temperature was approximately 70°. By way of control some of the untreated butter fat was irradiated immediately before feeding. At the end of the storage period the butter fat stored in the ice box was found palatable and unbleached; stored at room temperature it had a slightly rancid taste. The antirachitic potency of the various samples was tested using the curative type of experiment. Although, as already stated, this method does not reveal slight differences, it was deemed sufficiently accurate for the purpose desired. The butter fat, instead of being mixed with ration 2965, was measured out in small dishes and fed daily to the animals in addition to ration 2965. The animals were weighed at the beginning and end of the test, and consumption records were kept daily. The results, as shown in table 7, revealed marked variations among the experimental groups, but without definite relation to the treatment of the butter fat. In general, the results showed that antirachitic potency induced by irradiation is a stable property.

Since the discovery that ergosterol can be activated to a high degree of antirachitic potency, it immediately suggested itself

TABLE 7

Stability of vitamin D in butter fat to storage for seven months

LITTER NUMBER	RAT NUMBER	BUTTER FAT ADDITIONS TO RATION 2965	ADDITION DAILY	INITIAL WEIGHT	FINAL WEIGHT	AVERAGE GRAMS RATION 2965 CONSUMED	LINE TEST
			mgm.				
	8504	Irradiated, stored at room temperature	20	95	99	6.4	++
	8505	Irradiated, stored in icebox	20	102	105	7.9	+
66	8506	Non-irradiated, stored at room temperature, then irradiated	20	89	94	9.9	+
	8507	Non-irradiated, stored in icebox, then irradiated	20	91	98	7.2	?
	8520	Irradiated, stored at room temperature	20	86	94	9.7	++
	8521	Irradiated, stored in icebox	20	75	83	8.3	++
67	8522	Non-irradiated, stored at room temperature, then irradiated	20	84	97	8.8	+
	8523	Non-irradiated, stored in icebox, then irradiated	20	87	92	9.6	++
	8524	Non-irradiated, stored at room temperature, then irradiated	40	94	100	9.5	++
	8508	Irradiated, stored at room temperature	40	85	92	8.0	+++
	8509	Irradiated, stored at room temperature	40	83	92	8.6	+++
68	8510	Irradiated, stored in icebox	40	90	95	8.9	++
	8511	Irradiated, stored in icebox	40	88	98	9.0	+++
	8512	Non-irradiated, stored at room temperature, then irradiated	40	98	111	8.2	+
	8513	Non-irradiated, stored in icebox, then irradiated	40	84	92	6.3	++
	8514	Irradiated, stored at room temperature	80	79	80	7.3	+++++
	8515	Irradiated, stored at room temperature	80	82	77	5.8	+++
69	8516	Irradiated, stored in icebox	80	82	81	5.7	++++
	8517	Irradiated, stored in icebox	80	95	98	7.0	++++
	8518	Non-irradiated, stored at room temperature, then irradiated	80	109	117	10.6	++
	8519	Non-irradiated, stored in icebox, then irradiated	80	98	98	8.5	+++

that a food such as butter might be activated by dissolving therein the desired amounts of activated sterol. This would have the advantage that secondary reactions of ultra violet would be entirely eliminated, and the potency would be readily controllable. Calculations show that 1 ounce of irradiated ergosterol, depending upon its purity and treatment, would suffice for the activation of from 5 to 20 tons of butter, making it the equivalent of cod liver oil in antirachitic activity.

That this is a possible method of procedure is shown in table 8. For the experiments there was used ergosterol which was irradiated in ether solution. The ether was then dissipated at a low temperature and the residue dissolved in a small quantity of melted butter fat. This was then worked up with butter in the desired amounts.

In these experiments 10 mgm. of ergosterol were used for each pound of butter. This was obviously an excessive amount, but the feeding tests showed that the preparation was not of the potency usually attained. However, even though the maximum results were not secured, our data show that this amount made the butter twice as potent as cod liver oil and 80 times as potent as the original butter, an activity obviously excessive for practical purposes but nevertheless demonstrating possibilities.

It is to be emphasized that an excessive amount of vitamin D leads to harmful consequences, manifested by excessive calcium deposition in the soft body tissues. Fortunately, the amount necessary to produce these harmful results is of the order of 1,000 times the therapeutic dose (28). Nevertheless, the supplementation of foods with activated ergosterol needs to be carefully controlled. It suggests itself that this may be done in the case of butter by restricting the marketing of a preparation used for this purpose to dilute solutions or by limiting it to colored solutions. The ergosterol, for instance, might be dissolved in butter color. Our preliminary tests have not as yet shown that it is feasible to add the ergosterol solutions in such preparations previous to churning. Further work remains to be done. Both direct and indirect activation have their shortcomings, and it remains to be determined if vitamin D can always be satisfactorily fur-

TABLE 8

The antirachitic activation of butter by the addition of irradiated ergosterol and its comparison with cod liver oil

RAT NUMBER	ADDITION TO RATION 2965	PER CENT ASH IN FEMURS					GRAND AVERAGE	WIDTH OF METAPHYSIS	SIZE OF COSTO-CHONDRAL JUNCTION
408–411	5 mgm. daily cod liver oil	Femurs	47.27	43.50	31.05	45.31		Medium to narrow metaphysis (almost a wide rachitic metaphysis in rat 410)	Slight enlargement (pronounced enlargement in rat 410)
			46.36	43.65	30.55	45.08			
		Average	46.82	43.58	30.80	45.20	41.60*		
412–415	20 mgm. daily cod liver oil	Femurs	53.93	51.09	49.90	52.27		Normal	Normal
			53.62	51.31	50.43	51.95			
		Average	53.78	51.20	50.17	52.11	51.81		
416–419	200 mgm. daily untreated butter fat	Femurs	46.66	43.33	44.18	48.40		Medium to narrow metaphysis	Medium to very slight enlargement
			46.76	42.43	43.79	48.50			
		Average	46.71	42.88	43.99	48.45	45.51		
420–423	800 mgm. daily untreated butter fat	Femurs	54.23	51.58	53.17	53.25		Normal	Normal
			54.23	52.02	53.53	53.28			
		Average	54.23	51.80	53.35	53.27	53.16		
424–427	2.5 mgm. daily butter fat mixed with irradiated ergosterol	Femurs	45.34	41.78	48.75	49.82		Narrow metaphysis (medium metaphysis in rat 425)	Slight enlargement
			45.93	42.01	48.27	51.07			
		Average	45.64	41.90	48.51	50.45	46.62		
428–431	20 mgm. daily butter fat mixed with irradiated ergosterol	Femurs	51.09	50.73	†	51.78		Normal	Normal
			51.77	50.41		51.69			
		Average	51.43	50.57		51.74	51.25		

* Excluding rat 410 average is 45.20.

† Missing.

nished in the human diet by direct irradiation.   By their natural
limitations of activatability, due to paucity in content of ergos-
terol or to lack of surface, direct irradiation of many food mate-
rials would prevent all danger of hypervitaminosis.   Essentially,
the advisability of the fortification of foods with vitamin D is pri-
marily a problem of: first, what is the extent of the deficiency of
the human diet in vitamin D; and second, to what extent is it
absolutely essential that this deficiency be corrected?   The prac-
tical fortification of the human diet in vitamin D offers no essen-
tial difficulties.

<div align="center">CONCLUSIONS</div>

Curative and prophylactic techniques for the study of the
antirachitic potency of butter fat demonstrated the limitations of
the line test or so-called Johns Hopkins method.   For quantita-
tive work the prophylactic method, which makes use of the per-
centage of bone ash, does not leave open the opportunities for
error through mistaken judgment.   Its values are numerical
and quantitative.

June butter fat was found so low in vitamin D that 5 per cent of
the weight of a rickets-producing ration or an intake of about 400
mgm. per rat daily did not allow normal bone production.

June butter fat, however, did contain sufficient activatable
constituents so that treatment with ultra violet radiations under
suitable conditions made an intake of 20 mgm. of it per rat daily
equivalent in antirachitic potency to 400 mgm. of the untreated.

A sample of cod liver oil was found about 40 times as potent as
the June butter fat used in our experiments. .

The activating strength of two types of quartz mercury vapor
lamps, namely, the Cooper Hewitt B.Y. type and the Hanovia
Alpine Sun Lamp, was found to be approximately equal.

Using a film of fat 160 mm. thick, most of the activation by
exposure to the ultra violet radiations of our apparatus occurred
in the first 10 minutes of exposure or less.   The second 10 minutes
caused some additional activation, but the effect of the third 10
minutes was questionable.   After that, irradiation for a total of 2
hours or more caused no increase.   When the radiation was con-

tinued for a total of 16 hours, the activation originally induced or originally found in the butter fat was totally destroyed. This indicates that at least during the latter part of the activating process activation and destruction occur simultaneously. In practice, the intensity of the ultra violet treatment can, of course, be so adjusted that the exposure need be continued for only a fraction of a second, as is now done with cereals (30), because, in any event, maximum activation is neither necessary nor, probably, desirable.

The potency of irradiated butter fat was found stable to storage, and no deleterious effects of judicious irradiation were detected.

The practicability of activating butter by the introduction of irradiated ergosterol was demonstrated.

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SEASONAL VARIATIONS IN CERTAIN INORGANIC CONSTITUENTS OF DRY MILK PRODUCED IN NEW YORK AND WISCONSIN*

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The extension of nutritional and biochemical research during recent decades has brought into prominence the inherent quantitative variations of certain minor constituents of milk, both organic and inorganic. Although but meager data are as yet available directly connecting these inherent variations with the nutritive and dietetic properties of milk produced in different localities at different seasons of the year, the trend of many investigations is, nevertheless, toward a more careful study of such relationships. In the preparation of various milks designed for infant feeding, normal fluctuations in the constitution of milk which may to any degree affect the nutritive properties or physicochemical relationships during processing, are matters of concern.

Various inter-related studies carried on at this Laboratory have necessitated a determination of the limits of variation of certain inorganic constituents of the milk supply used for the preparation of a well known brand of dry milk[2] especially manufactured for infant and convalescent feeding. Inquiries from pediatrists, research institutions and individual investigators manifesting an interest in data of this character and its possible relationship to particular problems has prompted the compilation and publication of the data recorded herein.

Samples of dry milk made in representative milk producing areas of New York and Wisconsin were analyzed at monthly intervals throughout a period of one year. The test samples were

TABLE 1

Monthly variations in the ash content of dry milk produced in New York State territory

(Results expressed as percentages on moisture-free powder basis and on the ash basis)

MONTH	ASH	P₂O₅ Powder	P₂O₅ Ash	Cl Powder	Cl Ash	CaO Powder	CaO Ash	MgO Powder	MgO Ash	K₂O Powder	K₂O Ash	Na₂O Powder	Na₂O Ash	SO₃ Powder	SO₃ Ash	UNDETERMINED (ASH BASIS)	O-Cl EQUIVALENT (ASH BASIS)
January	7.625	2.103	27.58	1.031	13.52	1.718	22.53	0.186	2.44	2.000	26.22	0.516	6.76	0.170	2.23	1.77	3.09
February	7.639	2.103	27.52	1.073	14.04	1.720	22.51	0.183	2.39	1.980	25.91	0.524	6.86	0.120	1.56	2.37	3.16
March	7.608	2.100	27.60	1.049	13.79	1.723	22.64	0.188	2.45	1.925	25.30	0.492	6.45	0.181	2.37	2.51	3.11
April	7.531	2.110	28.01	1.025	13.61	1.720	22.82	0.188	2.49	1.900	25.22	0.474	6.29	0.184	2.44	2.19	3.07
May	7.594	1.972	25.96	1.015	13.36	1.713	22.55	0.186	2.44	2.010	26.45	0.581	7.65	0.172	2.26	2.64	3.01
June	7.634	1.903	24.89	1.038	13.58	1.688	22.08	0.187	2.44	2.150	28.11	0.591	7.73	0.170	2.22	2.01	3.06
July	7.709	1.914	24.82	1.023	13.27	1.690	21.90	0.184	2.37	2.160	28.01	0.588	7.64	0.180	2.33	2.66	3.00
August	7.785	1.949	25.03	1.027	13.17	1.702	21.86	0.192	2.46	2.210	28.38	0.574	7.37	0.181	2.32	2.38	2.97
September	7.801	2.050	26.28	1.007	12.90	1.705	21.85	0.198	2.53	2.130	27.30	0.569	7.29	0.182	2.32	2.44	2.91
October	7.810	2.052	26.27	1.091	13.95	1.707	21.85	0.188	2.39	2.080	26.63	0.552	7.06	0.178	2.27	2.72	3.14
November	7.765	2.061	26.54	1.005	14.10	1.705	21.95	0.183	2.36	2.010	25.89	0.552	7.11	0.179	2.31	2.91	3.17
December	7.630	2.105	27.59	1.022	13.39	1.720	22.54	0.183	2.39	2.010	26.34	0.560	7.34	0.170	2.23	1.22	3.02
Average	7.678	2.035	26.50	1.041	13.55	1.708	22.24	0.187	2.44	2.047	26.70	0.548	7.14	0.172	2.24	2.24	3.05

TABLE 2

Monthly variations in the ash content of dry milk produced in Wisconsin territory

(Results expressed as percentages on the moisture-free powder basis and on the ash basis)

MONTH	ASH	P_2O_5		Cl		CaO		MgO		K_2O		Na_2O		SO_3		UNDETERMINED (ASH BASIS)	O-Cl EQUIVALENT (ASH BASIS)
		Powder	Ash	Powder	Ash	Powder	Ash	Powder	Ash	Powder	Ash	Powder	Ash	Powder	Ash		
January	7.571	2.166	28.60	0.935	12.35	1.640	21.66	0.193	2.54	2.090	27.46	0.477	6.30	0.140	1.85	2.02	2.78
February	7.606	2.152	28.29	0.934	12.27	1.665	21.89	0.196	2.58	2.100	27.60	0.457	6.01	0.180	2.37	1.75	2.76
March	7.560	2.120	28.04	0.957	12.65	1.688	22.32	0.192	2.54	2.070	27.38	0.437	5.78	0.178	2.35	1.79	2.85
April	7.534	2.104	27.93	0.928	12.32	1.702	22.59	0.189	2.50	2.060	27.24	0.459	6.09	0.176	2.34	1.67	2.78
May	7.587	2.050	27.02	0.940	12.39	1.706	22.48	0.194	2.56	2.110	27.81	0.469	6.18	0.178	2.35	2.00	2.79
June	7.629	1.987	26.05	0.879	11.52	1.715	22.48	0.190	2.49	2.270	29.75	0.498	6.53	0.170	2.23	1.54	2.59
July	7.673	1.949	25.40	0.913	11.89	1.758	22.91	0.187	2.44	2.260	29.45	0.510	6.65	0.156	2.03	1.91	2.68
August	7.761	1.979	25.49	0.989	12.74	1.762	22.70	0.203	2.62	2.210	28.47	0.499	6.43	0.187	2.41	2.01	2.87
September	7.801	2.110	27.05	0.994	12.74	1.709	21.91	0.199	2.55	2.190	28.07	0.492	6.31	0.179	2.29	1.95	2.87
October	7.737	2.121	27.41	1.041	13.45	1.724	22.28	0.197	2.55	2.100	27.14	0.470	6.07	0.175	2.26	1.87	3.03
November	7.702	2.132	27.68	1.048	13.61	1.736	22.53	0.186	2.41	2.110	27.39	0.419	5.44	0.181	2.35	1.66	3.07
December	7.610	2.140	28.12	0.990	13.01	1.679	22.06	0.192	2.52	2.100	27.59	0.439	5.77	0.162	2.13	1.73	2.93
Average	7.647	2.084	27.25	0.962	12.58	1.707	22.32	0.193	2.52	2.139	27.97	0.469	6.13	0.172	2.25	1.80	2.82

composite samples representative of the milk received at commercial milk drying establishments located in these territories, and were selected on the same day of each month throughout the year. Tables 1 and 2 and charts 1 and 2 show the variations in total ash and certain of the mineral elements. Results are

MONTHLY VARIATIONS IN ASH CONTENT OF DRY MILK PRODUCED IN
NEW YORK AND WISCONSIN
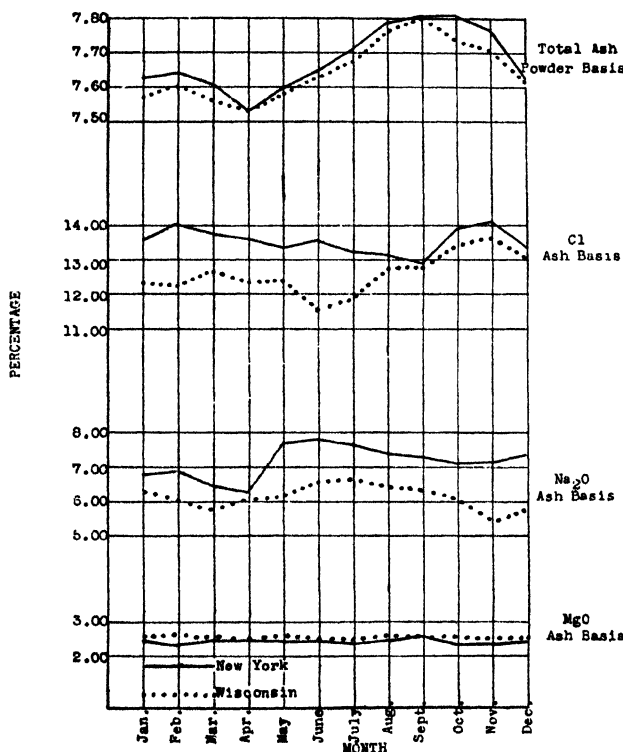
FIG. 1

expressed on the moisture-free powder basis and on the ash basis. The powder samples used for these analyses contained 12 per cent fat on the dry basis; the percentage of the various constituents may, therefore, be readily calculated for dry whole or skimmed milk, or for the reconstituted fluid products.

Although numerous analyses and special examinations for the minute traces of metals known to be normally present have been made on the milk produced in the New York and Wisconsin territories, the data as yet are too incomplete, with the possible

MONTHLY VARIATIONS IN ASH CONTENT OF DRY MILK PRODUCED IN
NEW YORK AND WISCONSIN
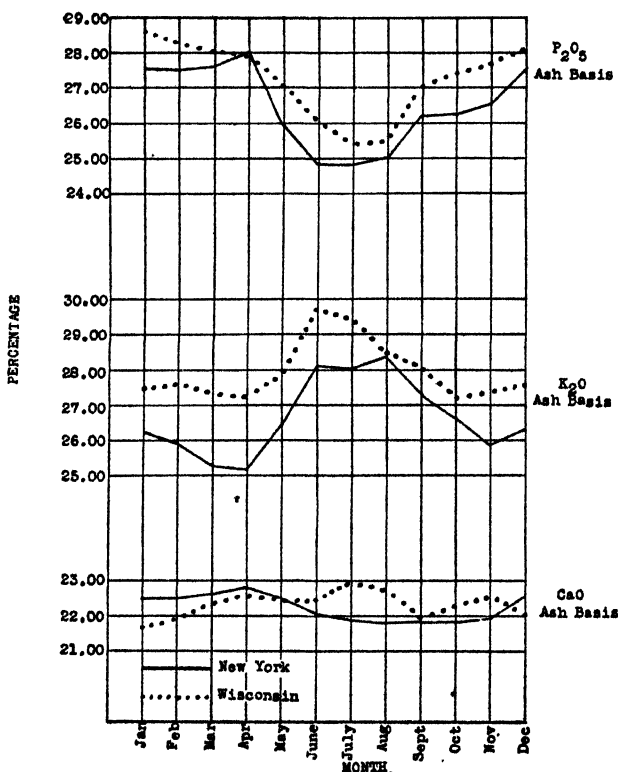
FIG. 2

exception of those pertaining to the iodine content, to warrant conclusions regarding the possible seasonal fluctuations.

The iodine content of the dry milk representative of the Winter and Summer production has been determined for the product made in each of the above localities. Composite samples were

prepared by selecting 12 ounces of the desiccated product representative of the day's production for each of 4 days on the same date of each month for the period from May to September inclusive as representing Summer production, and of the period from November to March as representing Winter production. The results from these analyses follow: The powder from the New York territory, Summer production, contained 145 parts of iodine per billion; the Winter production from the same territory contained 67 parts per billion. The powder from the Wisconsin territory, Summer production, contained 704 parts iodine per billion; the Winter production from the same territory contained 961 parts per billion.

SUMMARY

Analytical data are submitted showing the natural fluctuations in certain inorganic constituents of dry milk as affected by seasonal and territorial conditions in the States of New York and Wisconsin.

PROCEEDINGS OF THE TWENTY-FIFTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The Twenty-Fifth Annual Meeting of the American Dairy Science Association was held at Iowa State College, June 24, 25, and 26. There were approximately 250 in attendance. The industry was represented by men from all sections of the United States interested in the science and business of dairying. Members came from each of the three Pacific Coast States, from New England, from Middle Atlantic States, from Florida, Louisiana, South Carolina and other Southern States, and, of course, the North Central Section was especially well represented.

The program was a full one. The diversity of interests and activities of the dairy industry result in programs run more or less like a three ring circus. With meetings proceeding simultaneously on the economic phases of the industry, the manufacturing or product developments, and the production problems, one has difficulty in hearing all the papers in which he is interested.

There is an angle to these programs that seems somewhat unfortunate. You hear a paper read in which you are interested, one that you would like to go over carefully and digest thoroughly. But unlike the proceedings of some scientific societies, it has been the practice of the American Dairy Science Association not to publish the papers presented. Some of the reasons for this are: that many of the papers are merely a report of progress of investigations that will be published later in more detail; that some people do not want a paper published that will have to be briefed to the extent necessary for presentation in the time allowed on the program; then too, publication would perhaps mean the necessity of a review of the papers by the Program Committee prior to the meeting.

Those in attendance were enthusiastic in their praise of Professor Mortensen and his associates at Ames in the completeness of their arrangement for the comfort and amusement of the visi-

tors. The two banquets and a luncheon at Ames were greatly enjoyed. The dinner served on the lawn in the court of the splendid new Dairy Industry building at Ames, had such an attractive setting, that it will be long remembered by those who were so fortunate as to be present.

On the afternoon of the 26th the members were the guests of the Meredith Publishing Company, at Des Moines. They were conducted through the very modern and complete plant in which "Successful Farming" is published. Later we were taken to the Meredith farm near Des Moines, and shown their herd of registered Jerseys and served a delightful picnic supper. Mr. E. M. Harmon was in charge of ceremonies for the Meredith Publishing Company. On the following day the members were guests of the Cherry-Burrell Company and the Quaker Oats Company at Cedar Rapids. They were conducted through these large manufacturing plants and entertained at a dinner in the evening. Mr. J. A. McLean was in charge of the arrangements of this excursion for the Cherry-Burrell and Quaker Oats Companies.

At a business meeting of the association a committee for nomination of officers for 1931, consisting of Professors C. H. Eckles, R. B. Stoltz, and C. L. Roadhouse, submitted the following report:

> For president:
> Dr. H. A. Ruehe, University of Illinois.
> Dr. H. B. Ellenberger, University of Vermont.
> For vice-president:
> Prof. C. E. Wylie, University of Tennessee.
> Mr. F. W. Bouska, Beatrice Creamery Co., Chicago, Illinois.

The nominating committee calls attention to the provision of the constitution which makes possible additional nominations previous to November first by petition signed by not fewer than five members.

The following resolutions were presented and adopted.

WHEREAS the American Dairy Science Association has accepted the invitation of the Iowa State College, extended through the Dairy Industry Department, to hold its summer meeting of 1930 at the College; and

WHEREAS the Iowa Butter Manufacturers Association,
the Iowa Butter Makers Association,
the Iowa Ice Cream Manufacturers Association,
the Iowa Creamery Secretaries and Managers Association,
the Iowa State Brand Creameries Incorporated,
the Iowa State College Dairy Club,
the Meredith Publishing Company,
the Quaker Oats Company,
Cherry-Burrell Company and
Des Moines Milk Marketing Association,
have joined with the Faculty and Staff of the Dairy Industry Department and other departments of the college and the management of the Memorial Union, all under the leadership of Professor Mortensen in making our stay here most pleasant, comfortable, and informative.

Therefore, Be It Resolved by the American Dairy Science Association in convention assembled, that we tender to each individual and to each organization contributing to this splendid meeting and to the hospitality extended to our families and to ourselves—the thanks and appreciation of the members of the American Dairy Science Association individually and collectively.

WHEREAS the essential value of these meetings is dependent on the program arranged for, and the fulfilment of that program; and on the efficient conduct of the various meetings as well as on the work of the intervening year.

Therefore Be It Resolved that we hereby acknowledge our indebtedness to, and our appreciation of the excellent work of the program committee, the speakers, the officers, the editor of The Journal of Dairy Science, all of whom have contributed so much to that exchange of knowledge which makes for the progress of our science and industry and the inspiration of our members.

<div style="text-align:right">

H. E. VAN NORMAN
A. A. BORLAND
Committee on Resolutions.

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President Sherman discussed the desirability of some revisions in the constitution and by-laws of the association and asked Dr. C. H. Eckles to serve as Chairman of a committee to study and make recommendations for such revision. Dr. Eckles is to select his own committee.

There was some discussion as to whether the Divisions of the Association should be refunded fifty cents out or each membership fee, to defray costs of their annual meetings. The final action was that the parent association would continue to contribute up to twenty-five dollars a year toward the expenses of each Division pending recommendations of the Committee on Constitution.

Dr. L. A. Rogers moved that the Committee of the Association

appointed to act as an advisory committee with the National Research Council, be abolished. The motion was adopted.

Professor A. C. Ragsdale called attention to the National Dairy Improvement Contest sponsored by the St. Louis Chamber of Commerce, the outline of which follows:

NATIONAL DAIRY IMPROVEMENT CONTEST

Object: To promote better dairy management. The St. Louis Chamber of Commerce is interested in a National Dairy Improvement Contest on the following or a similar basis.

Unit: Considering the county as the unit and the county agent as the representative eligible to compete, the dairy extension departments of the various states would determine the winner from their state.

State Winners: The colleges would send in their winning report to be judged by a committee of competent judges.

Regional and National Winners: The judges would determine the regional winners, and from them select the sweepstakes winner.

Regions: There would be five regions—New England, Central, Central West, Southern, and Western.

Prizes: The winners would receive free trips to the National Dairy Exposition in St. Louis. They would also receive medals and certificates.

Basis of Awards: The awards would be made on the following basis:

	per cent
1. Analysis of problem........	30
2. Methods used.......	30
3. Results..................	40

Work along the following lines would be important—Dairy Herd Improvement Associations, Calf Clubs, Bull Associations, Feeding Work, Pastures, Dairy Management, and all things tending to promote dairying. The coöperation of civic groups on this program would add to its effectiveness.

Control of Contest· Such a contest should be conducted under the supervision of the American Dairy Science Association and the U. S. Bureau of Dairy Industry.

The St. Louis Chamber of Commerce would provide the trips for the regional winners. The medals and certificates would be provided by the National Dairy Association in the name of the American Dairy Science Association.

Professor Ragsdale moved:

"That a special committee, with power to act, be appointed to develop plans and methods and supervise the conduct of a National Dairy Development Contest with special awards to be made at the time of the 1931 and succeeding National Dairy Shows. This committee to coöperate with a representative of the St. Louis Chamber of Commerce and the National Dairy Association, in the furthering of this contest."

This motion was adopted and President Sherman appointed the following committee:

R. W. Balderson, National Dairy Council, chairman
A. C. Ragsdale, University of Missouri, secretary
E. S. Savage, Cornell University
J. H. McClain, U. S. Bureau of Dairy Industry
W. M. Regan, University of California
C. S. Rhode, University of Illinois
E. M. Harmon, Meredith Publishing Company

Attention was called to the fact that many States have very small representations in the membership of the American Dairy Science Association. Some twenty-five of the States have fewer than five members. Those States having the greater number of members are:

New York	45	Michigan	16
California	38	Missouri	16
Illinois	32	District of Columbia	15
Ohio	21	Indiana	14
Wisconsin	17	New Jersey	12
Minnesota	17	Massachusetts	11
Pennsylvania	17	Iowa	11
		Vermont	10

Dr. Roadhouse of California told how he had written to men in commercial work in his State who were interested in the scientific aspects of the dairy industry, and explained to them the desirability of their being members of the American Dairy Science Association and getting The Journal of Dairy Science. He had a·very favorable response and secured a number of new members. Dr. Eckles said he had been successful in securing new members in Minnesota in much the same way.

The executive committee voted in favor of holding the 1931 Annual Meeting on the Pacific Coast.

The Pacific Coast members had already arranged a tentative plan and schedule for the meeting and tour and had printed copies for distribution at Ames. According to these plans the meeting would be held on July 1 and 2 at Davis, California. Two different pre-convention trips are planned commencing

June 26 and continuing up to the time of the program to be followed by another tour lasting until July 16.   There can be no doubt that the Pacific coast meeting will be outstanding as a combination of the program with sight-seeing and educational tours.

The following program was presented:

GENERAL SESSION

June 24, 1930

President J. M. Sherman, presiding

Registration—Memorial Union.

Call to order—(Great Hall, Memorial Union).

Address of Welcome.   R. M. Hughes, President, Iowa State College.

President's Address.   J. M. Sherman, Cornell University.

Announcements.

The economic situation of the dairy industry.   Roy C. Potts, Bureau Agricultural Economics, U. S. D. A.

Significance and use of body measurements of dairy cattle.—W. W. Swett, Bureau of Dairy Industry, U. S. D. A.

The relation of plant operation to flavors in milk.   H. A. Ruehe and P. H. Tracy, University of Illinois.

The mineral composition of bones of dairy cattle as influenced by age and nutrition.   L. S. Palmer, University of Minnesota, and W. N. Neal, University of Florida.

Some factors affecting the development of nutritional anemia in the white rat.   W. B. Nevens, University of Illinois.

Viability of Lacto-bacillus Acidophilis at different temperatures.   M. J. Prucha, University of Illinois.

Packaging cheese without processing.   L. A. Rogers, Bureau of Dairy Industry, U. S. D. A.

The nervous reaction of animals on a deficiency diet (Film).   J. S. Hughes and H. W. Cave, Kansas State College.

DAIRY MANUFACTURERS AND BACTERIOLOGY

C. L. Roadhouse, Chairman, Section Dairy Manufactures, presiding

Studies in the use of chemical disinfectants—A. C. Fay, Kansas State College

A study of churn contamination.   Part I. The growth of microörganisms on the wood of the churn.   M. S. Libbert, University of Arkansas.

The importance of solubility of milk powders.   J. C. Marquardt, N. Y. State Experiment Station.

The solubility of copper in milk under various conditions and its estimation by an electrochemical method.   H. T. Gebhardt and H. H. Sommer, University of Wisconsin.

A comparison of the influence of iodized milk and of potassium iodide administered directly on the size and iodine content of the thyroid gland of rats.   W. E. Krauss and C. F. Monroe, Ohio Experiment Station.

The chemical nature of the fatty materials in buttermilk. E. W. Bird, Iowa State College.

Studies in the manufacture of sweet-curd or baker's cheese. L. M. Thurston, University of West Virginia.

Modified Babcock tests for fat in ice cream. L. K. Crowe, University of Nebraska.

Some observations on the basic viscosity of ice cream mixes. J. C. Hening, N. Y. State Exp. Station.

Influence of the source of fat and serus solids on overrun and quality in ice cream. J. M. Jensen, Michigan Agricultural College.

A visit to the Dairy Industry Building and the Dairy Farm.

Mixer at the Dairy Industry Building.

DAIRY PRODUCTION AND OFFICIAL TESTING

H. B. Ellenberger, Chairman, Section of Dairy Production, presiding

Influence of water bowls on water consumption of dairy cows. C. Y. Cannon, Iowa State College.

Influence of sunshine on the growth and on the milk of dairy heifers. T. M. Olson, South Dakota Agricultural College.

Effect of delayed milking and pressure on milk secretions. W. E. Petersen and T. Rigor, University of Minnesota.

The effects of storage on the composition of root crops. K. S. Morrow, University of West Virginia.

Results of recent tabulations of dairy herd improvement records. J. C. McDowell, Bureau of Dairy Industry, U. S. D. A.

The therapeutic effect of cod liver oil and ultra-violet light upon the nutrition of calves. S. E. Bechdel, Pennsylvania State College.

Energy and nitrogen metabolism of dairy calves. Samuel Brody, University of Missouri.

A study of the comparative efficiency of electrically operated cooling tanks versus ice in the cooling of milk. J. H. Frandsen, Massachusetts Agricultural College.

The effect of chemical sterilizers on the rubber parts of milking machines. E. H. Parfitt, Purdue University.

Vitamin A as a limiting factor in the growth of dairy calves that receive no roughage. W. M. Regan, University of California.

A visit to the Dairy Industry Building and the Dairy Farm.

Mixer at the Dairy Industry Building.

June 25

DAIRY PRODUCTS AND BACTERIOLOGY

C. L. Roadhouse, Chairman, Section of Dairy Manufactures, presiding

A test for fat in condensed and evaporated milk and ice cream. W. D. Swope, Pennsylvania State College.

Relation of temperature in ice cream to the distribution of certain constituents in its solid and liquid phases. V. C. Cole, University of California.

The effect of dilution on the titratable acidity of cows' milk. Jules Menos and
   H. H. Sommer, University of Wisconsin.
Quantitative changes in the microflora during the manufacture of butter. S. T.
   Coulter, University of Minnesota.
Relation of feathering and heat stability of cream to fat clumping due to homo-
   genization. F. J. Doan, Pennsylvania State College.
The relation between the age of cream when delivered at the buying station and
   the quality of the resulting butter. V. C. Manhart, Purdue University.
Surface taint in butter. H. A. Derby and B. W. Hammer, Iowa State College.
The electric charges on milk fat globules and their relation to various dairy phe-
   nomena. G. C. North and H. H. Sommer, University of Wisconsin.
Section meeting, committee reports, business.

DAIRY PRODUCTION AND OFFICIAL TESTING

H. B. Ellensberger, Chairman, Section of Dairy Production, presiding

Cottonseed meal as the sole concentrate for dairy heifers. Earl Weaver, Okla-
   homa Agricultural College.
The individuality of the four quarters of the udder. C. W. Turner, University of
   Missouri.
The comparative feeding value of immature versus mature soybean hay for milk
   and fat production. J. H. Hilton, Purdue University.
The contribution of the Bowlker Hybrid herd to our knowledge of dairy cattle
   breeding. W. W. Yapp, University of Illinois.
A study of the sugar content of the blood of dairy animals. Ralph Hodgson and
   W. H. Riddell, J. S. Hughes, Kansas State College.
Preparation and methods of analysis of bones of dairy cattle. W. N. Neall,
   University of Florida, and L. S. Palmer, University of Minnesota.
Value of grinding roughage. L. H. Fairchild, Letz Manufacturing Company.
The number of daughters necessary to prove a sire. Jay L. Lush, Iowa State
   College.
Section meeting, committee reports, business.

EXTENSION SECTION

G. A. Williams, Chairman, Dairy Extension Section, presiding

Improving the quality of dairy products. J. B. Parker, Bureau of Dairy Indus-
   try, U. S. D. A.
Comparison of unit requirements for milk production in northeastern, central,
   and southwestern districts. C. H. Willoughby, University of Florida.
The value of testers' conferences.
   Discussion led by A. C. Baltzer, Michigan State College.
                          E. N. Shultz, Iowa State College.
                          Ed. Hansen University of Minnesota.
                          C. R. Gerhart, Pennsylvania State College.
Feeding Schools.
   Discussion led by H. R. Searles, University of Minnesota.
                          Ivan McKellip, Ohio State University.
                          E. A. Gannon, Purdue University.

Demonstration of a mechanical device for rapid calculations in balancing rations. C. M. Long, Blue Valley Creamery Company.

GENERAL PROGRAM

H. C. Jackson, Vice-President, presiding

The protein problem in dairy feeding. W. J. Fraser, University of Illinois.

Determination of the hemoglobin content of the blood of dairy animals. H. J. Brooks and J. S. Hughes, Kansas Agricultural College.

Vitamin A content in the milk of different breeds. I. L. Hathaway, H. P. Davis, University of Nebraska.

An experimental review of Tillmann's method for detecting the neutralization of milk and cream. Kenneth Week and H. H. Sommer, University of Wisconsin.

A method for improving the quality of butter of a state without excessive expenditures. L. C. Thomsen, University of Wisconsin.

The use of cocoa, chocolate liquor, and prepared chocolate syrups in the manufacture of chocolate ice cream. W. J. Caulfield, Kansas State College.

Some bacteriological and temperature studies in milk plants. C. W. Leete, Bureau of Dairy Industry, U. S. D. A.

The use of chemical deodorants in cream for buttermaking. W. H. Martin and C. L. Smith, Kansas State College.

The effect of uneven intervals of milking upon the lactose and chloride content of milk. C. L. Roadhouse and J. L. Henderson, Univerity of California.

Speed of dasher and scraper as affecting the quality of ice cream. E. L. Reichart, University of Nebraska.

Development of butter culture. R. Farmer and B. W. Hammer, Iowa State College.

Business meetings of sections.

EXTENSION SECTION

June 26

G. A. Williams, Chairman, Dairy Extension Section, presiding

Standardized rules for Official Testing of all dairy breeds. E. L. Anthony, Michigan State College.

The data necessary to prove a purebred sire. Warren Gifford, University of Missouri.

The use of herd improvement records as a means of proving dairy bulls. J. C. McDowell, Bureau of Dairy Industry, U. S. D. A.

Locating and retaining proved sires.

Discussion led by J. A. Simms, Connecticut Agricultural College.

E. N. Shultz, Iowa State College.

E. J. Perry, Rutgers College.

E. T. Wallace, Purdue University.

M. J. Regan, University of Missouri.

DAIRY PRODUCTS AND BACTERIOLOGY

J. M. Sherman, President American Dairy Science Association, presiding

Yeast and mold content of strawberry ice cream. P. K. Downs, University of Nebraska.

Correlation between the annual butterfat production and the feed cost of dairy cows. C. W. McIntyre.

The effect of feeding potatoes to dairy cows on the production and quality of milk and butterfat. J. R. Dice, North Dakota Agricultural College.

An organism rapidly increasing the acid number of butter. R. V. Hussong and B. W. Hammer, Iowa State College.

The effects of rice by-products upon the fatty acid of butterfat. M. S. Libbert and H. E. Dvorachek, University of Arkansas.

The relation between the per cent of fat in the cream and the fat loss in the buttermilk. E. W. Bird and M. Mortensen, Iowa State College.

Sweetened frozen cream for the manufacture of ice cream. Walter V. Price, University of Wisconsin.

Properties of casein for paper coating. R. W. Bell, Bureau of Dairy Industry, U. S. D. A.

The transition points of some milk fats. G. A. Richardson, University of California.

The precipitation of gelatin in chocolate ice cream. Floyd E. Kurtz, Bureau of Dairy Industry, U. S. D. A.

Influence of the acidity of the butter culture on the quality of butter. Chris Jensen, North Dakota Agricultural College.

Observations on churn sanitation. Henry Morrison, University of Minnesota.

<div align="center">COLLEGE INSTRUCTION PROGRAM</div>

<div align="center">*E. L. Anthony, Michigan Agricultural College, presiding*</div>

Do we need a dairy education. A. D. Burke, Oklahoma Agricultural College.

College Dairy Herds, size, number of breeds, utilization. Earl Weaver, Oklahoma Agricultural College.

How much time should be given to teaching dairy cattle judging. W. E. Petersen, University of Minnesota.

Inspection and judging trips, value, time allowed. J. R. Dice, North Dakota Agricultural College.

How essential is laboratory work and how much time should it take. A. A. Borland, Pennsylvania State College.

Can good manufacturing instruction be given without the operation of a college creamery. E. L. Reichart, University of Nebraska.

Maintaining the student interest. Elmer Hansen, Iowa State College.

Teaching dairy feeds by the use of problems. R. B. Becker, University of Florida.

Lecture, recitation, or problem. L. K. Crowe, University of Nebraska.

Is there a place for a market milk course. H. Macy, University of Minnesota.

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